3-25-91

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAR 25 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

Chemical: Vinclozolin
Chem. #: 113201
EFGWB #: 91-0184
DP Barcode: 158397
Case #: 2740

MEMORANDUM

SUBJECT: Review of Phase IV Package for Vinclozolin

TO: Amy Rispin, Chief
Science Analysis and Coordination Staff
Environmental Fate and Effects Division (H7507C)

THRU: Henry Jacoby, Chief
Environmental Fate and Ground Water Branch
Environmental Fate and Effects Division (H7507C)

Paul J. Mastradone, Chief, Review Section 1
Environmental Fate and Ground Water Branch
Environmental Fate and Effects Division (H7507C)

FROM: Arnet W. Jones, Agronomist
Environmental Fate and Ground Water Branch
Environmental Fate and Effects Division (H7507C)

Enclosed is the Phase IV review package for List B chemical vinclozolin, case no. 2740 (chemical no. 113201).

1. Use Patterns

According to the LUIS report of Nov. 6, 1990, vinclozolin (3-[3,5-dichlorophenyl]-5-ethenyl-5-methyl-2,4-oxazolidine-dione) has terrestrial food and non-food uses. A fungicide used to control Botrytis spp. in fruits, vegetables, and ornamentals, vinclozolin can be applied at a maximum rate of 1.04 lb a.i./A. Up to six applications can be made during a growing season resulting in total of 6.24 lb a.i./A applied per crop cycle.

2. Environmental Fate Data Requirements

The Phase IV package received by EFGWB contained eight summaries, four of which related to studies which were reviewed (see DERs attached):

- Photodegradation in water (161-2)

HJ
3-25
489

- Aerobic soil metabolism (162-1)
- Confined accumulation in rotational crops (165-1)
- Accumulation in fish (165-4)

Four of the summaries covered new studies which were submitted for the first time with the Phase IV package. The studies appear to be reviewable based on the summaries. These studies will be reviewed in Phase V:

161-1 Hydrolysis
161-3 Photodegradation on soil
162-2 Anaerobic soil metabolism
163-1 Leaching/adsorption/desorption

The following environmental fate data requirements remain unfulfilled:

161-1 Hydrolysis
161-2 Photodegradation in water
161-3 Photodegradation on soil
162-1 Aerobic soil metabolism
162-2 Anaerobic soil metabolism
162-3 Anaerobic aquatic metabolism
163-1 Leaching/adsorption/desorption
163-2 Laboratory volatility (see Other Issues below)
164-1 Terrestrial field dissipation
165-1 Confined rotational crops
165-4 Bioaccumulation in fish

According to the registrant's Phase III response, studies in support of most of these data requirements have been or will be conducted. In its Phase II response and in correspondence submitted with its Phase III response, the registrant indicated that anaerobic aquatic metabolism (162-2) data are not applicable for vinclozolin. However, EFGWB now requires acceptable anaerobic aquatic metabolism data for chemicals with terrestrial food crop and non-food crop uses because of the potential of the chemical to reach aquatic systems via runoff. Acceptable anaerobic aquatic metabolism data can substitute for anaerobic soil metabolism data, but the opposite is not possible.

The following environmental fate data requirements are reserved:

161-4 Photodegradation in air (see Other Issues below)
163-3 Field volatility (see Other Issues below)
164-5 Long-term terrestrial field dissipation
165-2 Field accumulation in rotational crops
165-5 Bioaccumulation in aquatic non-target organisms
166-1 Ground water - small scale prospective
166-2 Ground water - small scale retrospective
166-3 Ground water - large scale retrospective
167-1 Surface water - field runoff
167-2 Surface water - surface water monitoring
201-1 Droplet size spectrum
202-1 Drift field evaluation

Refer to the attached table for a summary of the environmental fate data requirements.

3. Environmental Fate Summary

None of the environmental fate data requirements have been fully satisfied. However, some information pertaining to the fate of vinclozolin in the environment is known based on the following studies, all of which are supplemental.

In water at pH 2-3, with acetone added as a sensitizer, vinclozolin undergoes photodegradation with a half-life of less than 4 hours. There was no observable photolysis of unsensitized vinclozolin in the test solution at pH 1.94 over 46 days.

In two aerobic soil metabolism studies carried out at 20 ± 2°C in a Neuhofen (German) soil judged to be comparable to U.S. soils, radiolabeled vinclozolin degraded with a half-life of 53 days. In two experiments carried out at 25 ± 2°C in the same soil, radiolabeled vinclozolin degraded with half-lives of 34.7 and 40.8 days. In a Pfungstadt (German) soil (there was insufficient data to determine whether this soil compares favorably with U.S. soils), radiolabeled vinclozolin underwent biphasic degradation with half-lives of 5.4 and 6.9 days during the first 14 days at 20 and 25°C, respectively. From day 14 until the end of the experiment (day 120), the rate of degradation slowed with half-lives of 46.2 and 49.5 days reported for the two respective temperatures.

In a supplemental study for confined accumulation in rotational crops, recovered ^{14}C for soybeans was 3.162 and 0.635 ppm for 60 and 365 day aged soils, respectively. For wheat, total ^{14}C recovered was 2.632 and 1.104 ppm for 60 and 365 day aged soils, respectively. Total ^{14}C in carrots for the 60 and 365 day aged soils was 23.533 and 1.859 ppm, respectively. Except for carrots, main levels of total ^{14}C were found in the roots.

In a supplemental fish accumulation study, bluegill sunfish had maximum bioconcentration factors of 106X, 321X, and 241X for edible, non-edible, and whole fish, respectively. After 14 days of depuration, ^{14}C residues declined by 97-98% from maximum observed concentrations.

4. Other Issues

In its Phase III response the registrant submitted waiver requests for photodegradation in air (161-4) and laboratory volatility (163-2) data. The waiver request for laboratory volatility data cited vinclozolin's vapor pressure (3.4×10^{-6} mbar at 25°C, or 2.6×10^{-6} mm Hg) as justification for the data waiver. Because volatilization is a possible route of dissipation in the environment, EFGWB requires acceptable laboratory volatility data on all compounds whose vapor pressure exceeds 10^{-6} mm Hg. Therefore, laboratory volatility data for vinclozolin will be required. The data requirements for photodegradation in air (161-4) and field volatility (163-3) will be reserved pending results of the laboratory volatility study.

DATA EVALUATION RECORD

STUDY IDENTIFICATION:

Study 1: Ohnsorge, U. 1980. Unsensitized photolysis of vinclozolin in aqueous solution. MRID no. 53092. BASF Lab Communication No. 888.

Study 2: Huber, Beutel, and Ohnsorge. 1978. Sensitized photolysis of ^{14}C-vinclozolin in water. MRID no. 136373. BASF Lab Report No. 1599.

Amendment to Study 2. Huber, R. 1988. Amendment to Lab Report No. 1599: Sensitized photolysis of ^{14}C-vinclozolin in water. MRID no. 414710-07.

REVIEWED BY:

Arnet W. Jones, Agronomist
Review Section I, EFGWB

Signature: Arnet W. Jones

Date: MAR 25 1991

APPROVED BY:

Paul J. Mastradone, Ph.D. Chief
Review Section I, EFGWB

Signature: Paul J. Mastradone

Date: MAR 25 1991

TYPE OF STUDY: Photolysis in Water (161-2)

CONCLUSIONS:

1. The studies provide supplemental information regarding the aqueous photolysis of vinclozolin.

2. In study 1 (MRID no. 53092) there was no observable photolysis of unsensitized vinclozolin in the test solution at pH 1.94 over a period of 46 days of continuous exposure to light at wavelengths >280 nm. When acetone was added as a sensitizer, vinclozolin photolyzed rapidly with 80% degradation after 76 hours.

3. In study 2 (MRID no. 136373), where acetone was used as a sensitizer in a solution at pH 2-3, vinclozolin exhibited half-lives of 3.6 and 3.8 hours by TLC and HPLC, respectively, following continuous exposure to light at wavelength >280 nm. TLC analysis detected one metabolite with R_f value close to that of metabolite E (see attached); there were no other photoproducts in concentrations >10% of the applied radioactivity.

4. Both studies were conducted at very low pH values (pH 1.94 in the unsensitized study; pH 2-3 in the sensitized study) since vinclozolin undergoes hydrolysis above pH 2. Aqueous photolysis data at these low pH values are not useful for predicting vinclozolin behavior in the environment.

4

5. The data presented do not reflect adequately the role, if any, of aqueous photolysis as a route of vinclozolin dissipation in the environment. Therefore, a new aqueous photolysis study should be conducted at the most hydrolytically stable pH between 5 and 9. The standard evaluation procedure (SEP) for aqueous photolysis studies should be used for guidance.

MATERIALS AND METHODS:

Study 1 (MRID no. 53092): Prior to the aqueous photolysis study, an experiment was carried out to determine the stability of vinclozolin in aqueous solution. Twelve buffer solutions ranging from pH 0 to pH 5.0 were prepared using 0.1N H_2SO_4 and citrate. Non-radiolabeled vinclozolin (>99.5% pure) was added to methanol to prepare a stock solution containing 5 mg/mL. 10 uL of vinclozolin stock solution was injected into 10 mL of each buffer to achieve a vinclozolin concentration of 5 mg/kg. At time zero and on days 2, 5, 14, and 23, 1 mL samples were drawn from each lot and partitioned with 5 mL of n-hexane. Duplicate injections of the organic layers were analyzed by GC. The mean values were compared to a standard solution of 1 ug/mL vinclozolin in hexane.

After determining that vinclozolin is stable at pH 2, an unsensitized aqueous photolysis study was conducted. Vinclozolin (3.00 mg) was dissolved in 0.60 mL methanol and added to a solution of 1.74 g H_2SO_4 in 1.500 L of water in a 1:1 photoreactor to yield a concentration of 2 mg/kg. Air was bubbled through the solution, the pH of which was 1.94. Dark controls were made from 250 mL of this solution. A cooler insert and high pressure mercury arc lamp TQ 150 (original Hanau) were mounted and irradiation of the test solution begun. The test solution was irradiated with wavelengths >280 nm. The air leaving the irradiated flask was bubbled through ethanol and analyzed for vinclozolin.

Duplicate 2 mL samples were removed from the test solution beginning at time 0 and continuing at sampling intervals up until 1106 hours (46 days). Samples were partitioned with 5.00 mL hexane and analyzed by GC, "mostly by duplicate injections of 5.0 uL." The results of duplicate injections were averaged. Dark controls were analyzed at 24, 166, and 316 hours.

When no vinclozolin photolysis was observed after 1106 hours of irradiation, 22.0 mL of acetone was added to the solution and irradiation was continued.

Study 2 (MRID no. 136373): Ring-labeled ^{14}C-vinclozolin (529.9 ug in 4.75 mL acetone) was added to 255 mL of aerated acidified water (pH 2-3). This solution (2.02 ppm vinclozolin in 1.83% acetone) was transferred to an irradiation flask. A stream of air was passed over the solution surface and subsequently passed through two traps, one of which contained 0.1N H_2SO_4; the other contained scintillation cocktail for trapping radioactive volatiles. The solution was stirred

magnetically and the temperature was kept at 20 ± 2°C.

The solution was irradiated for 5 hours with a high pressure mercury arc TQ 150 lamp which emitted light at wavelengths >280 nm. Samples were drawn at time 0 and at 1-hour intervals. A dark control study also was performed with samples being drawn only after 5 hours. Dark controls were analyzed by the method used for sample 7 (see following paragraph).

Samples 1-6 (time 0 through 5 hours; 2mL each) were extracted with ethyl acetate. The organic phase was assayed for radioactivity by LSC, with further analysis by HPLC and TLC. The aqueous phase was analyzed by LSC. Sample 7 (260 mL) was extracted with n-hexane and ethyl acetate. The n-hexane and ethyl acetate phases were analyzed by LSC, TLC, HPLC, and GC/MS. In addition, the ethyl acetate phase was subjected to alkaline hydrolysis to determine the portion of the radioactivity which consisted of the intact 3,5-dichloroaniline moiety of the molecule.

REPORTED RESULTS:

Study 1 (MRID no. 53092): The results of the preliminary experiment indicated that vinclozolin is stable in aqueous solution at pH ≤ 2.0. Hydrolysis occurred at higher pH values.

In the main study, there was no observable photolysis of vinclozolin in the test solution over a period of 46 days (see attached table). Recoveries of vinclozolin ranged from 94.7% (415 hours) to 105.4% (50 and 76 hours). There was no observable degradation of vinclozolin in dark controls.

When acetone was added to the photolysis solution following 1106 hours of irradiation, 19.8% of the initial vinclozolin concentration remained in the photoreactor following an additional 76 hours of irradiation.

Study 2 (MRID no. 136373): TLC and HPLC data indicated vinclozolin half-lives of 3.6 and 3.8 hours, respectively. TLC analysis detected one metabolite with R_f value close to that of metabolite E; there were no other photoproducts which exceeded 10% of the applied radioactivity.

In the analyses of sample 7, 45% of the applied radioactivity was in the n-hexane fraction; 53% was in the ethyl acetate fraction, and 2% was found in water. HPLC and MS indicated that the major portion of the radioactivity in the ethyl acetate consisted of the formyl derivative U (see structures - attached).

Alkaline hydrolysis of an aliquot of ethyl acetate extract confirmed the stability of the 3,5-dichloroaniline moiety indicating no cleavage of the ring and no chlorine transfer.

TLC analysis of the control detected only parent vinclozolin.

DISCUSSION:

1. Both studies were conducted at low pH values (pH 1.94 and pH 2-3 for studies 1 and 2, respectively) because vinclozolin is not subject to hydrolysis at low pH. Because aqueous photolysis is a possible route of pesticide dissipation in the environment, studies should be carried out at the most hydrolytically stable pH between 5 and 9 since pH values outside this range are not commonly found in the environment.

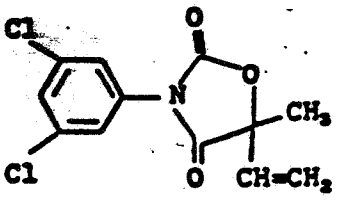
2. Neither glassware nor solutions were sterilized in the studies. However, because of the very low pH of the solutions, it is very unlikely that organisms, and hence biological degradation, were a factor in study 2 where vinclozolin degradation occurred.

3. Both studies were reviewed previously (study 1 - Dec. 16, 1980; study 2 - Aug. 27, 1979; copies of these reviews are attached). Although the data requirement for aqueous photolysis was judged to be met at that time, EFGWB will require acceptable aqueous photolysis data at pH values which are likely to be found in the environment.

4. The supplement to study 2 contained additional information including data tables, statistical treatment of data, graphs, and a proposed photolytic pathway for vinclozolin degradation. There are no data in the supplement which affect EFGWB's conclusions regarding the study.

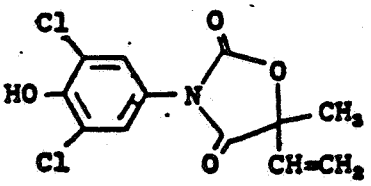
7

FIGURE III



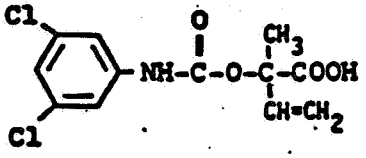
BAS 352 F, vinclozolin

3-(3.5-dichlorophenyl)-5-ethenyl-5-methyl-1.3-oxazolidine-2.4-dione



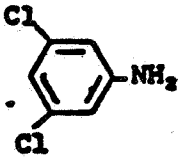
A

3-(3.5-dichloro-4-hydroxyphenyl)-5-ethenyl-5-methyl-1.3-oxazolidine-2.4-dione



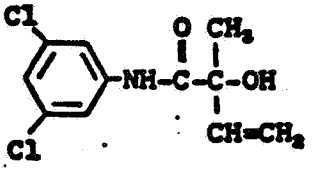
B

3.5-dichlorophenyl-carbamic acid(1-carboxy-1-methyl)2-propenyl ester



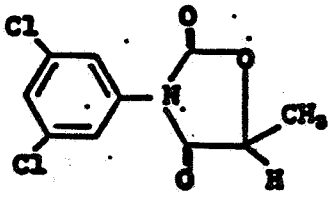
D

3.5-dichloroaniline



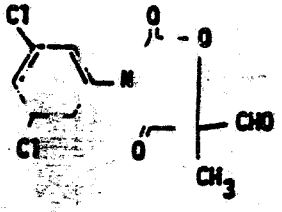
E

N-(3.5-dichlorophenyl)-2-hydroxy-2-methyl-3-butenoic acid amide



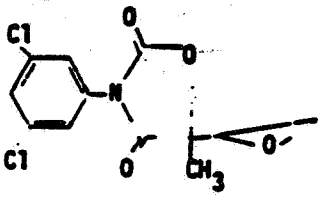
S

3-(3.5-dichlorophenyl)-5-methyl-1.3-oxazolidine-2.4-dione



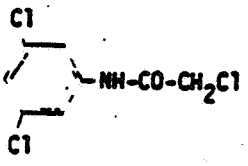
U

3-(3.5-dichlorophenyl)-5-formyl-5-methyl-1.3-oxazolidine-2.4-dione



V

3-(3.5-dichlorophenyl)-5-oxiranyl-5-methyl-1.3-oxazolidine-2.4-dione



DCAD

N-(3.5-dichlorophenyl)-chloro-acetamide

8

D. Luck 5-04-81 1

(5)

2.00 ml samples were drawn in duplicates up to 1106 hrs (46 days) and kept in the dark until a batch had accumulated. The samples were partitioned with 5.00 ml hexane and the organic layers analysed by GC, mostly by duplicate injections of 5.0 µl. The individual results were averaged and listed below.

Hours irradiation	% Recovery of vinclozolin	Raw data in attachment
0	100	3a
3	99.3	3a
24	99.3	3a
50	105.4	3b
76	105.4	3b
166	106	3b
199	98.4	3c
247	99.0	3c
312	99.7	3c
382	95.1	3d - e
415	94.7	3d
484	98.4	3d - e
577	98.3	3d - e
674	96.9	3d - e
946	99.6	3d - e
1106	101.5	3d - e
Hours dark control:		
24	99.3	3a
166	103.6	3b
316	100	3c

9

10 (6)

3.0 DISCUSSION OF DATA

BASF Wyandotte Corporation submitted additional data as requested in EFB review of 3/25/80. Data were filed under Accession No. 243393, Registration No. 7969-53, dated 12/2/80. The title of each study and data discussion are shown below:

3.1 Photolysis Study REVIEW OF DEC 16, 1980

The company submitted additional data to augment the photolysis study previously reviewed, in an attempt to elucidate the fate of vinclozolin in aqueous solution; the light source used; and a profile of the soil used in the soil surface photolysis study. The following studies were submitted:

(a) Stability of Vinclozolin in Aqueous Solution

Ten ml solution containing 5 mg vinclozolin per ml methanol was injected into 10 ml of each buffer medium of various pH ranging from 0 to 5 at final concentration equivalent to 5 mg/kg of vinclozolin.

After periods of 0, 2, 5, 14 and 23 days, one ml samples were drawn from each lot and partitioned with 5 ml hexane. The organic layers were analyzed by GC.

Test results showed that percent recovery of Vinclozolin varied according to pH and time elapsed from start to analysis. It was apparent, however, that Vinclozolin showed the greatest stability at pH 2 where percentage recovery was near 100% and was independent of sampling time.

(b) Unsensitized Photolysis of Vinclozolin

Three mg vinclozolin dissolved in 0.6 ml methanol, was added to a solution of 1.74g H_2SO_4 in 1.5 H_2O in a photoreactor while bubbling air through the mixture giving a final concentration equivalent to 2 mg/kg of an acidic solution (pH 1.94). A mercury lamp TQ 150 was mounted and the solution was irradiated (>280 nm) for a period of up to 1106 hours (46 days).

Two ml samples were drawn at certain irradiation periods and partitioned with 5 ml hexane and the organic layers analyzed by GC.

Test results showed that vinclozolin was stable in aqueous solution at pH ≤ 2. [redacted]

Vinclozolin at concentration 2 mg/kg in acidic aqueous solution (pH 1.94) is not susceptible to unsensitized photolysis by artificial sunlight of wavelengths >280 nm within 46 days.

(c) Soil Profile Used in the Soil Surface Photolysis Study

As requested in EFB review of 3/25/80, BASF company submitted a profile of the soil used in the soil surface photolysis study. These are: Loamy sand soil, OM 2.5%, pH 6.8, CEC 10 m Val/100g, bulk density 1.4 g/ml, sand 2000-200M 67%, sand 200-20 M 16%, silt 20-2 M 7%, and clay < 2M 10%.

10

CHEMICAL: Ronilan, Vinclozolin, 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione (formerly BAS 353F, BAS 352(04)F, 83 258)

FORMULATION: Technical

CITATION: Huber, Beutel and Ohnsorge. (1978). Sensitized Photolysis of ^{14}C-Vinclozolin in Water (unpublished report prepared by BASF Wyandotte Corp., Parsippany, NJ, Report No. 1599)

TRADE SECRET CLAIM: Yes

REASON FOR REVIEW: Registration of Ronilan

REVIEWED BY: M. Edwards, Environmental Scientist
Enviro Control, Inc. Rockville, MD

DATE OF REVIEW: August 27, 1979

TEST TYPE: Sensitized Photolysis Study in Water

CONCLUSIONS:

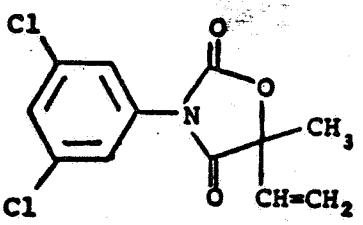
1. No formal conclusions were presented by the authors. The data show that in the presence of a sensitizer 2 ppm of labeled vinclozolin in water is rapidly degraded under simulated sunlight, with a half-life of approximately 4 hours. The only major photo-product found was derivative U, 3-(3,5-dichlorophynyl)-5-formyl-5-methyl-1,3-oxazolidine-2,4-dione, which accounted for approximately 40% of the original radioactivity.

2. The reviewer is impressed with the procedures and protocols of this study and considers it scientifically valid. The study meets EPA guidelines for the conduct of photodegradation determinations in water, and provides the required data of half-life estimates, identification of major photoproducts(< 10% of the original pesticide) and material balances.

3. The major problem with the study, in the reviewer's judgment, is in determining the relevance of the findings as they relate to the degradation of the compound under actual environmental conditions. This is due to the conditions under which the study was conducted (2% acetone sensitized, pH 2-3).

MATERIALS AND METHODS:

A carbon-14 labeled preparation of vinclozolin was used for the experiments in the study:



BAS 352 F - Vinclozolin
3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-1,3-oxazolidine-2,4-dione

A quantity of 529.9 µg of the test compound in 4.75 ml of acetone was added to 255 ml of aerated acidified bidistilled water (pH 2-3). This solution (2.02 ppm labeled Vinclozolin in 1.83% acetonic water) was transferred to the irradiation vessel/sunlamp apparatus, a high pressure mercury arc TQ 150 with a cooler envelop. A stream of air was passed over the solution and through two traps, one filled with 0.1N H_2SO_4 and the second filled with scintillation cocktail for the collection of radioactive volatiles. The outside of the irradiation flask was covered with aluminum foil. Temperature was maintained at 20± 2°C, by external cooling with water, and the solution was magnetically stirred.

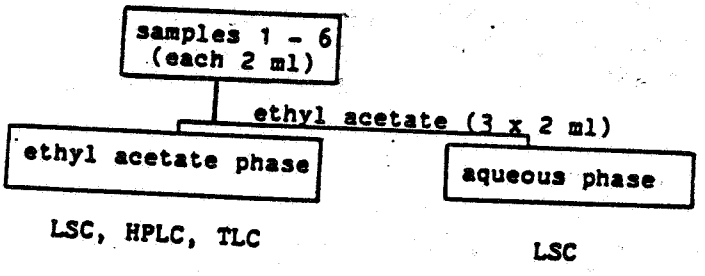
The solution was irradiated for five hours, with samples drawn according to the schedule below. A dark control experiment was also conducted with samples of the unexposed solution taken after five hours and analyzed in the same manner as Sample 7 in the irradiation experiment (see discussion below).

Samples from irradiation of ^{14}C-vinclozolin solution

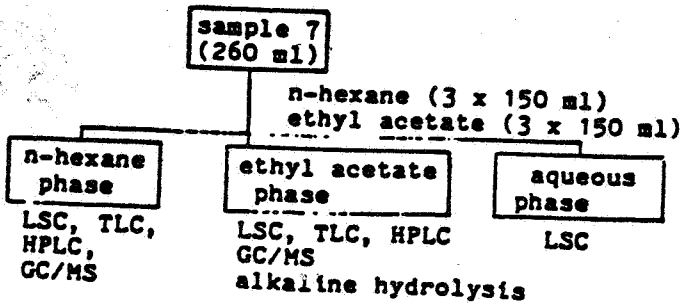
Sample no.	hours of irrad.	ml drawn
1	0	2
2	1	2
3	2	2
4	3	2
5	4	2
6	5	2
7	5	~260

The extraction and analysis of Samples 1-7 are outlined schematically below. Briefly, Samples 1-6 were extracted with ethyl acetate, and Sample 7 was extracted with ethyl acetate and n-hexane. Radioactivity measurements were determined through liquid scintillation counting (LSC), with further investigation of the extracts conducted using thin-layer chromatography (TLC) spotted against reference compounds (see Figure 1). Radioactive spots on developed plates were located using a TLC-scanner, and quantified by coupling the scanner to a multichannel analyzing system. Extracts were analyzed using high pressure liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS). Alkaline hydrolysis was used with Sample 7 to determine the portion of the radioactivity in the ethyl acetate phase which consisted of the intact 3,5-dichloroaniline moiety.

Samples No. 1-6



Sample No. 7



REPORTED RESULTS

RADIOACTIVITY in TRAPS

No radioactivity was found in the H_2SO_4-filled trap, and in the second trap filled with liquid scintillation cocktail radioactivity was only found after 5 hours, representing 0.2% of the applied radioactivity.

HALF-LIFE of LABELED-VINCLOZOLIN (Figures I & II)

The half-life of labeled-Vinclozolin was determined by plotting the concentrations of the compound in solutions 1-6 over time. Based upon TLC and HPLC data half-lives were found to be 3.6 and 3.8 hours, respectively. TLC showed a major photoproduct at an R_f value close to metabolite E, which increased as the parent compound decreased. No other photysis products were found which exceeded 10% of the originally applied radioactivity.

EXAMINATION OF SAMPLE 7 (Figure III)

Analysis of extracts from Sample 7 which revealed 45% of the radioactivity in the n-hexane phase, 53% in the ethyl acetate phase and 2% of the radioactivity in the water phase was taken as an indication that extremely polar products were not being formed. Characterization of the n-hexane extract indicated the formation of a non-polar product identified as epoxide V, which did not exceed 4% of the total radioactivity. TLC analysis of the ethyl acetate extract from Sample 7 revealed an unknown photoproduct accounting for approximately 40% of the originally applied radioactivity. This product had an R_f value just above that of metabolite E. HPLC and MS indicated that the major portion of the radioactivity in the ethyl acetate phase was due to the formyl derivative U. Derivatives S and D were also found, but were considered to have been thermally formed in the gas chromatograph, as it was felt that these products would have otherwise been found in the n-hexane extract. Two other products (3,5-dichlorophenyl-isocyanote and 3,5-dichloropyruvic acid anilide) representing approximately 5% of the total radioactivity in the irradiated solution were also found. Mass spectra data are presented in the original report for the degradation products described above.

Alkaline hydrolysis, applied to an aliquot of ethyl acetate, confirmed the stability of the 3,5-dichloraniline moiety, indicating to the authors that neither chlorine transfer nor cleavage had occurred.

CONTROL EXPERIMENT

Radioanalysis of the extracts from the unexposed control sample after 5 hours showed that 99.6% of the original radioactivity was present in the n-hexane phase, 1.4% in the ethyl acetate phase and 0.1% in the water. TLC indicated the presence of only the parent compound.

DISCUSSION:

1. This study was conducted following two preliminary studies, one showing that cold Vinclozolin remained stable for 30 days during unsensitized irradiation in water, and the second study indicating that the half-life of cold Vinclozolin in water under sensitized conditions (acetone) was less than 10 hours.

2. The procedures and protocols followed in this study are considered well conceived and are impressive to the reviewer. This includes the extensive analytical techniques (TLC, HPLC, GC/MS) used to characterize degradation products and the presentation of raw data including HPLC and MS spectral data. A control experiment was run, and a spectral energy distribution indicating that wavelengths exceeded 280 nm was provided. Carbon-14 recoveries, in the reviewer's judgement, were good.

3. The use of a single carbon-14 preparation of Vinclozolin is appropriate in the reviewer's opinion, expecially in light of the alkaline hydrolysis data indicating stability of the 3,5-dichloroaniline moiety, the good carbon-14 recoveries, and the absence of any unidentified photoproducts representing more than 10% of the initial radioactivity.

4. The authors' statement that under the conditions of the study and in the absence of light, labeled-Vinclozolin is stable, is, in the reviewer's judgement, strongly supported by the data.

5. The authors speculated that epoxide V observed in the hexane fraction is the unstable precursor to the ethyl acetate soluble major photolysis product U. The authors further stated that from these findings, it becomes obvious that photolysis of Vinclozolin in water is oxidative in nature, with the primary attack probably initiated at the ethenyl group of labeled Vinclozolin. The process is thus one of photochemical epoxydation. The reviewer considers this to be a reasonable explanation, but suggests that the authors could have gone a step further and made hypotheses regarding potential reaction mechanisms and the role of the acetone sensitizer. It is recognized however, that the generation of such hypothesis is beyond the scope of the original objective.

6. The use of a low pH (2-3) was justified by the authors as necessary to prevent hydrolysis of the parent compound prior to photolysis. Unfortunately, in the reviewer's opinion, this makes it very difficult to determine what role, if any, photolysis may play under actual environmental conditions. The fact that no hydrolysis was observed in the control suggests to the reviewer that a pH closer to neutral could have been chosen. The authors did not hypothesize as to how pH changes might be expected to change photolysis rates.

43-16

Figure I

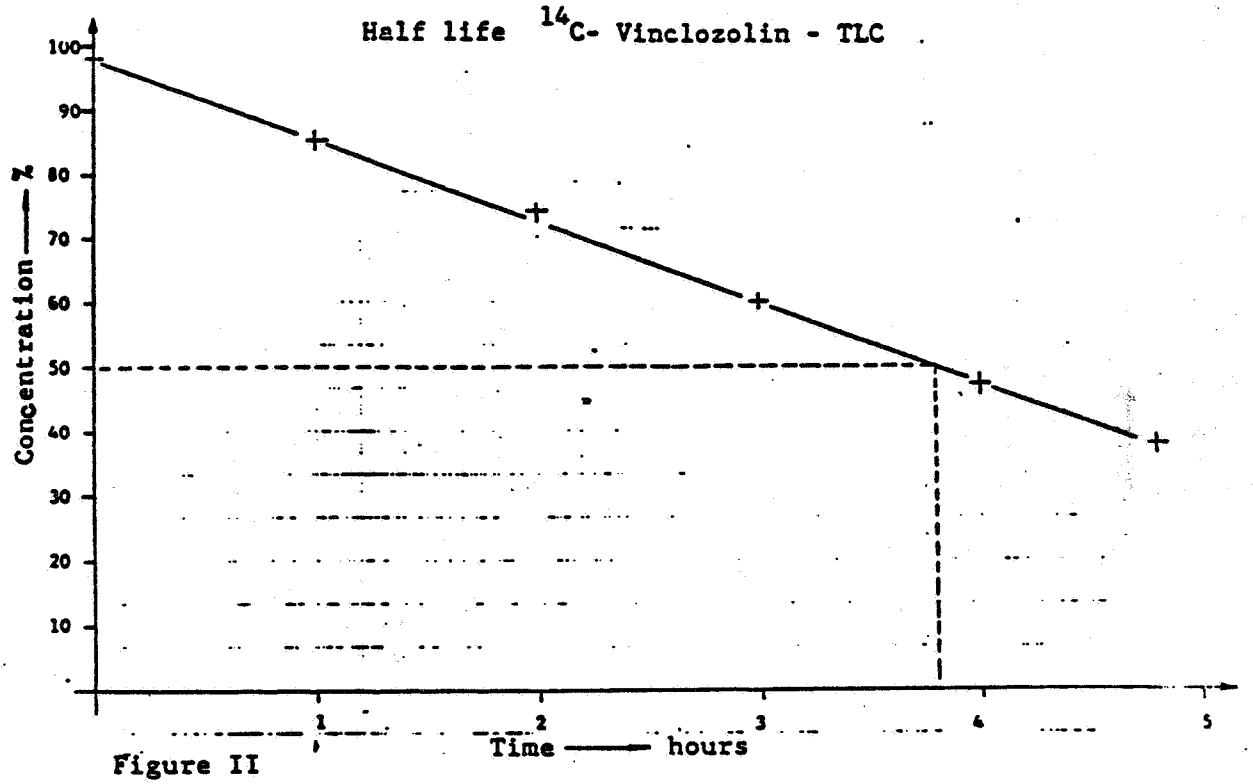


Figure II

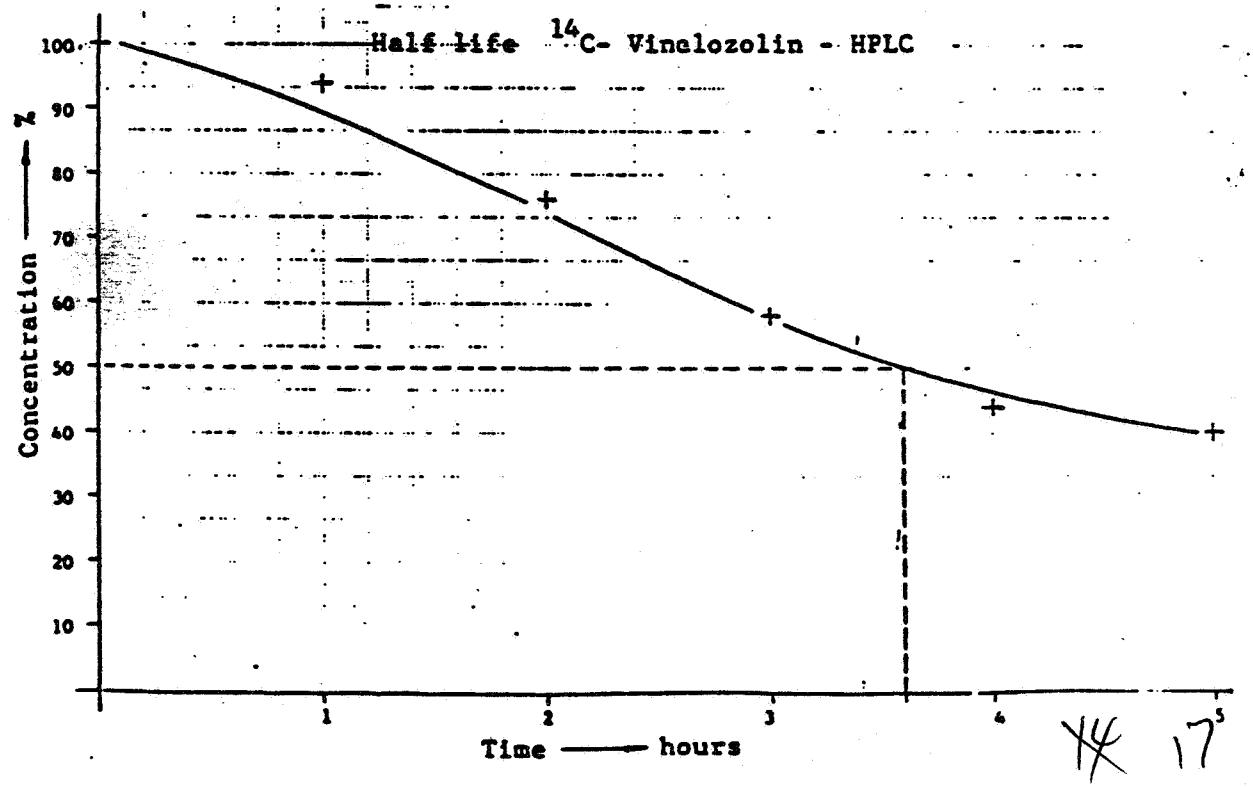
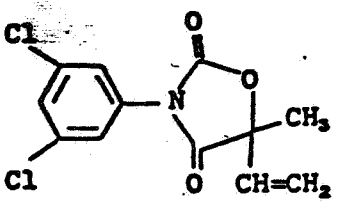
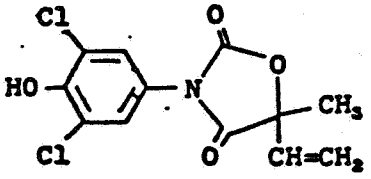


FIGURE III



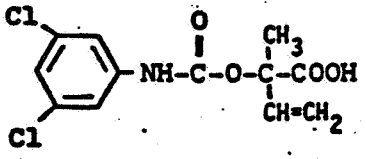
BAS 352 F, vinclozolin

3-(3.5-dichlorophenyl)-5-ethenyl-5-methyl-1.3-oxazolidine-2.4-dione



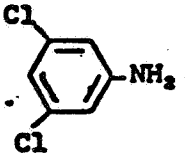
A

3-(3.5-dichloro-4-hydroxyphenyl)-5-ethenyl-5-methyl-1.3-oxazolidine-2.4-dione



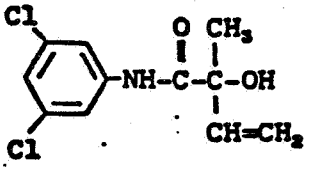
B

3.5-dichlorophenyl-carbamic acid(1-carboxy-1-methyl)-2-propenyl ester



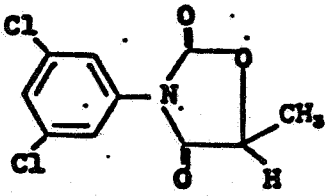
D

3.5-dichloroaniline



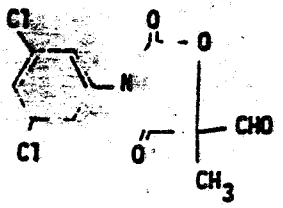
E

N-(3.5-dichlorophenyl)-2-hydroxy-2-methyl-3-butenoic acid amide



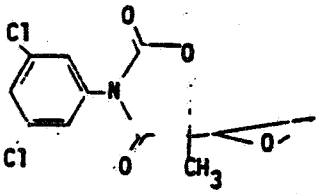
S

3-(3.5-dichlorophenyl)-5-methyl-1.3-oxazolidine-2.4-dione



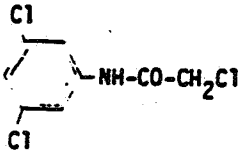
U

3-(3.5-dichlorophenyl)-5-formyl-5-methyl-1.3-oxazolidine-2.4-dione



V

3-(3.5-dichlorophenyl)-5-oxiranyl-5-methyl-1.3-oxazolidine-2.4-dione



DCAD

N-(3.5-dichlorophenyl)-chloro-acetamide

DATA EVALUATION RECORD

STUDY IDENTIFICATION:
Huber, R. and S. Otto. September 1978. Uptake of Aged ^{14}C-Vinclozolin (BAS 352F-^{14}C) Soil Residues by Rotational Crops; No. 1589. MRID# 00136385.

REVIEWED BY:

Elizabeth A. Resek, Chemist
Environmental Chemistry, Review Section 1
OPP/EFED/EFGWB

Signature _____
Date MAR 20 1991

APPROVED BY:

Paul Mastradone Ph.D., Chief
Environmental Chemistry, Review Section 1
OPP/EFED/EFGWB

Signature _____
Date MAR 20 1991

TYPE OF STUDY: Confined Rotational Crop

CONCLUSION:

EFGWB concludes that the study (previously accepted 3/25/80 and rotation intervals set; see DISCUSSION) does not satisfy present data requirements for confined rotational crop. The study is judged supplemental and cannot be made acceptable upon the submission and review of additional information. A new study is required. Soil residue analyses and storage stability data are required.

Based on the results of supplemental data, recovered ^{14}C for soybeans was 3.162 and 0.635 ppm for 60 and 365 day aged soils, respectively. For wheat, total ^{14}C recovered was 2.632 and 1.104 ppm for 60 and 365 day aged soils, respectively. Total ^{14}C in carrots for the 60 and 365 day aged soils was 23.533 and 1.859 ppm, respectively. Except for carrots, main levels of total ^{14}C were found in the roots.

MATERIALS AND METHODS:

Chemical ^{14}C-vinclozolin (specific activity 9.23 mCi/mMol) was used in the study. The soil used was a loamy sand with the following characteristics: 83% sand, 7% silt, 10% clay; pH 6.8; CEC 10 mVal/100g; bulk density 1.4 g/ml; and 2-6% OM. Prior to seeding of rotational crops, 5 kg portions of soil were aged for 60 and 365 days. During the aging periods, soil was maintained at 40%

maximum water capacity in the dark at 20 ± 2 C. After each respective aging period (60 and 365 days), black plastic pots (13 cm i.d., 10 cm high) were filled with 1 kg soil and appropriate crop seed (summer wheat, soybeans or carrots). The emerging plants were kept in a growth chamber at 20 ± 2 C with artificial light at 12-hour periods. Plants were watered and thinned as needed.

Thirty days after seeding and at harvest time (98-147 days) samples were removed and kept at what appears to be -20 C until analysis. the following plant parts were separated before analyses: soybeans (beans, pods, leaves, roots), wheat (grains, ears, stalks, roots), carrots (leaves, roots). At the 30 day interval only, above ground plant parts were sampled.

Radioactivity of solid and liquid extracts was determined by combustion and LSC quantitation of $^{14}CO_2$. Samples were extracted with methanol. Fractions were further partitioned using various liquid/liquid partitions to divide the extracts into various phases. Extracted and paritioned phases were analyzed by TLC using several solvent systems. HPLC was used to determine whether the absorbed residues included the intact dichloroaniline moiety.

REPORTED RESULTS:

Total radioacativity taken up by the rotational crops can be seen in column 7 of Table I. Recovered ^{14}C for soybeans was 3.162 and 0.635 ppm for 60 and 365 day aged soils, respectively. For wheat, total ^{14}C recovered was 2.632 and 1.104 ppm for 60 and 365 day aged soil, respectively. Total ^{14}C in carrots for the 60 and 365 day aged soil was 23.533 and 1.859 ppm, respectively. Except for carrots, main levels of total ^{14}C are found in the roots. The methanol extractable radioactivity (column 9) differed considerably depending on tissue investigated. TLC results (see attachments) show predominantly parent (BAS 352 F) detected. There is recovery of metabolite F (N-3,5-dichlorophenyl)-2,3,4-trihydroxy butanoic acid amide) in soybean leaves.

DISCUSSION:

EFGWB concludes that the study does not satisfy data requirements for confined rotational crop. The study cannot be made acceptable, and therefore, a new study is required. The registrant should note the following points:

1. Residues in soil should be analyzed at the time of treatment, at the time of planting the rotational crop, and at the time of harvest of the rotational crop.

2. Storage stability data are required.

3. Total ^{14}C recovery was reported using LSC, and it appeared from most TLC scans that residues recovered were in the form of parent vinclozolin. However, one scan showed metabolite F recovered, which was identified but not quantified. Residues should be characterized.

4. EFGWB previously accepted the present study (3/25/80) and along with an additional study (accepted 4/27/82) concluded that the confined rotational crop data requirement was fulfilled. Rotation intervals were established. Since the study is not acceptable under present Guidelines, the rotation intervals previously established are no longer valid. EFGWB recommends, however, that the intervals be used until a new study is submitted and judged acceptable by EFGWB.

Rotation Interval for Vinclozolin
Rotational crop data permits rotation only to the following and only when indicated total pounds active ingredient applied per acre have not been exceeded through the previous season:

1. Lettuce may be planted 6 months after treatment not exceeding 12 lb a.i./A.

2. Squash may be planted 2 months after treatment not exceeding 9 lb a.i./A.

3. Corn may be planted 2 months after treatment not exceeding 9 lb a.i./A with use of only the corn grain for food and /or feed purposes.

4. Spring wheat may be planted 9 months after treatments not exceeding 8 lb a.i./A.

VINCLOZALIN Sh# 113201

Page_____ is not included in this copy.

Pages 22 through 30 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DATA EVALUATION RECORD

STUDY IDENTIFICATION:

Clark, J.R. and S.N. Adamsbaum. December 1981. Uptake of BAS 352 F-^{14}C (Ronilan) by Rotational Crops Under Field Conditions. BASF Wuandotte Corporation, parsippany, New Jersey. MRID# 094617.

REVIEWED BY:

Elizabeth A. Resek, Chemist
Environmental Chemistry, Review Section 1
OPP/EFED/EFGWB

Signature _____
Date MAR 20 1981

APPROVED BY:

Paul Mastradone Ph.D., Chief
Environmental Chemistry, Review Section 1
OPP/EFED/EFGWB

Signature _____
Date MAR 20 1981

TYPE OF STUDY: Accumulation on Rotational Crops

CONCLUSION:

EFGWB concludes that the study (previously excepted 4/27/82) does not satisfy data requirements for confined rotational crop under present Guidelines, but may be used however, for supplemental information for the field accumulation on rotational crops data requirement. For confined rotational crop studies, a confined, controlled system is required. Also, following soil treatment pesticides should be aged under aerobic conditions in the soil for a time approximating the anticipated agricultural practice. Note however, that a field accumulation on rotational crops study is not required under present use patterns for vinclozolin.

MATERIALS AND METHODS:

The chemical, ^{14}C-vinclozolin (specific activity 3.86 mCi/mMol, radiochemical purity 99%) was used in the study. The soil used was a silt loam with the following characteristics: 68% silt, 12% sand, 21% clay; pH 5.5; CEC 9.5; and 2.3% OM.

Lettuce seedlings were transplanted into 4 rows in a 16 ft^2 section of a 4 x 8 ft plot. Each plot contained 20 plants. Four plots were treated with ^{14}C-vinclozolin; two received 0.75 lb a.i./A and two received 1.0 lb a.i./A per application (total of 3 applications

per plot). Lettuce was harvested and discarded. After removal of target crop soil was spaded to a depth of 15 cm. Representative rotational crops (see Appendix 1) were planted 20, 30, 60 and 90 days after the last ^{14}C-vinclozolin application.
In most cases, both plant and soil samples were analyzed at three or more intervals. Three 12 inch soil cores were removed at each crop sampling period and devided into 4 inch sections. Appropriate sections from the 3 cores were combined, mixed and analyzed for total radioactivy by LSC. Plant samples were collected at random from the individual plots at various time periods, frozen with dry-ice, and kept frozen until analyzed.

REPORTED RESULTS:

The data in Table I show ^{14}C equivalents in rotational crops. No plant sample analyzed had a total ^{14}C residue level greater than 0.07 ppm of ^{14}C-vinclozolin equivalents. The 0-4 inch soil residue levels varied from over 0.7 ppm to 0.2 ppm during the sampling period. None of the soil residues appeared to leach beyond the top 8 inches of soil.

Monthly rainfall and temperatures were reported in Appendix 2.

DISCUSSION:

EFGWB concludes that the study (previously excepted 4/27/82) does not satisfy data requirements for confined rotational crop under present Guidelines, but may be used however, for supplemental information for the field accumulation on rotational crops data requirement.

The registrant should note the following deficiencies:

1. A confined, controlled system is required (i.e. use of pots or subplots which contain bottoms).

2. Following soil treatment, pesticides should be aged under aerobic conditions in the soil for a time approximating the anticipated agricultural practice.

3. Storage stability data are required.

The study is only supplemental for the accumulation on rotational crops data requirement due to the following deficiencies:

1. Field accumulation studies should be conducted in at least two different sites. Soil type at one of the test sites should be the same as that used in the confined study.

2. Following soil treatment, pesticides should be aged under aerobic conditions in the soil for a time approximating the anticipated agricultural practice.

3. Storage stability data are required.

4. There should be characterization of parent and degradates in addition to the reporting of total ^{14}C.

It should be noted that the field accumulation study is not presently required under use patterns for vinclozolin.

VINCLOZALIN Sh# 113201

Page_____ is not included in this copy.

Pages 34 through 43 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DATA EVALUATION RECORD

STUDY IDENTIFICATION:

Otto, S. 1976. Investigations into the degradation of BAS 352 F (vinclozolin) in soil. MRID no. 88288. BASF Lab Report No. 1388.

Reviewer's note: This DER should be reviewed in conjunction with DERs for two other aerobic soil metabolism studies (MRID nos. 136376 and 136377)

REVIEWED BY:

Arnet W. Jones, Agronomist
Review Section I, EFGWB

Signature: Arnet W. Jones

Date: MAR 25 ...

APPROVED BY:

Paul J. Mastradone, Ph.D. Chief
Review Section I, EFGWB

Signature: Paul J Mastradone

Date: MAR 25 1991

TYPE OF STUDY: Aerobic Soil Metabolism (161-2)

CONCLUSIONS:

1. The study provides supplemental information regarding the aerobic soil metabolism of vinclozolin.

2. In an aerobic soil metabolism study carried out for 240 days at 20 ± 2°C using a Neuhofen (German) soil judged to be comparable to U.S. soils, radiolabeled vinclozolin degraded with a half-life of 53 days. Degradation products identified as metabolites B, D, and E were identified by TLC (see structures attached).

3. The study will be considered supplemental because frozen storage stability data were not presented and because there was no confirmatory method used for identification of degradates. Refer to the DER for MRID no. 136376 for additional information required for meeting the aerobic soil metabolism data requirement.

MATERIALS AND METHODS:

35 mg of ring-labeled vinclozolin (purity >98%; specific activity 9.23 mCi/mMol) was added to 5 kg of Neuhofen (German) sandy loam soil (pH 6.8; 2.6% organic matter). The soil was transferred to a 5L tube and the water content was adjusted to 15.7% (40% of maximum water holding capacity). The tube was covered with a watch glass and placed in a temperature controlled room at 20±2°C. The water content was maintained constant by weighing and, when necessary, adding distilled water. Soil samples of 10-20 g each were taken on days 0, 7, 14, 30, 60, 90, 120, 150, 180, 210, and 240 and stored in closed flasks at -21°C until

analyzed.

An aliquot (200-800 mg) of each sample was combusted in order to determine the total radioactivity in the soil at the time of sampling. The $^{14}CO_2$ produced was absorbed in scintillation cocktail and measured by LSC. Soil (10 g) was extracted by methanol (50 mL) for 1 hour mechanically. Samples were filtered and the residue washed with methanol. Portions of extracted soil were combusted to determine the amound of radioactive material which was not extracted. Sufficient methanol was added to the extract to reach a specific (unspecified) volume. An aliquot of this was removed to determine the amount of radioactivity extracted. Methanol extracts were evaporated to 5 mL, the concentrate was dissolved in water, and this was brought to a volume of 100 mL. The aqueous mixture was extracted three times with 50 mL ethyl acetate. The extracts were combined, dried over Na_2SO_4, and adjusted to volume (200 mL) with ethyl acetate. Aliquots (1 mL) of the aqueous phase and organic phase were dissolved in 10 mL of Unisolve I and analyzed by LSC.

Ethyl acetate extracts were dissolved in methanol and aliquots were spotted on a TLC plate which was developed with two solvent systems (chloroform and chloroform:glacial acetic acid [95:5]). Compounds were identified against standards spotted on TLC plates. After development plates were scanned and exposed to X-ray film for 3 days. Radioactive zones were scraped from the plates for quantitation.

After 240 days a 200 g soil sample was taken to characterize residues, which were not extracted with methanol. Soils containing these residues were treated with acid and/or base in order to precipitate out humic substances. These were analyzed for radioactivity or hydrolyzed in boiling 5N KOH. The base hydrolysis solution was distilled into H_2SO_4, extracted with chloroform, and treated with chloracetyl chloride. Chloracetyl chloride forms the chloracetyl analogue of 3,5-dichloroaniline, a degradation product of vinclozolin. GLC was used to analyze for this analogue. Treatment of methanol-insoluble residues is outlined schematically in study pp. 24-25 (attached).

Measurement of $^{14}CO_2$ Formed During Vinclozolin Degradation

Soil spiked with ring-labeled vinclozolin (7 ppm) was placed in a closed container. Soil was added to a wash bottle with a glass frit and flushed with CO_2-free air. Air leaving the wash bottle, air passed through a scrubber containing 1N H_2SO_4 for absorption of acidic and basic compounds. The air then was passed through a scrubber containing scintillation cocktail.

REPORTED RESULTS:

Methanol Extractable Residues

The decline in vinclozolin (BAS 352) residues is shown in Table 8. From these data, the study reports a half-life of the parent compound of

45

"about 45 days." Table 8 shows that the concentrations of metabolite B reached a peak of 0.71 ppm on day 30 and declined to 0.13 ppm at day 240. The study summary indicates that the formation of metabolite B from vinclozolin is reversible (see proposed metabolic pathway attached).

Table 1 shows the distribution of ^{14}C residues calculated as vinclozolin equivalents. The total radioactivity, measured at the beginning of each sampling interval, ranged between 6.46 and 7.17 ppm. Radioactivity in the non-methanol extractable fraction increased from 0.14 ppm at time zero to 5.56 ppm at day 210. Methanol-extractable radioactivity decreased from 6.94 ppm at time zero to 1.16 ppm at day 210. Residues in the ethyl acetate fraction decreased from a high of 5.33 ppm at time zero to 0.91 ppm at day 210. The material balance ranged between 78 and 110% (calculated by the reviewer - see Discussion for details).

Table 8 shows that vinclozolin (BAS 352) residues declined from a high of 5.11 ppm at day 7 to 0.33 ppm at day 210. Metabolite B residues peaked at 0.71 ppm (day 30) and gradually declined to 0.13 ppm at day 240.

Degradation products D and E were identified by TLC (two solvent systems) but their concentrations were not reported in the study narrative.

Non-methanol Extractable Residues

Two humic acid precipitates (HS1 and HS4 - see study pp. 24-25) were examined to determine whether the dichloroaniline moiety of the vinclozolin molecule could be identified (i.e. adsorbed to the organic matter). Of the radioactive material extracted from the two humic substances, 28 and 44% were identified as dichloroaniline, respectively. The author reports that dichloroaniline may be further metabolized (and hence unidentified) or bound so tightly to the soil organic fraction that it cannot be extracted.

Measurement of $^{14}CO_2$ Formed During Vinclozolin Degradation

After 45 days, 0.53% of the radioactivity applied to the test soil was recovered in the scrubber as $^{14}CO_2$. The study author reports that "the rate of CO_2 formation remained constant over the period of time covered."

DISCUSSION:

1. The time interval between sampling and extraction and analysis should be specified. The study reports that soil samples were taken at sampling dates "and stored in closed flasks at -21°C until analyzed." The study summary (MRID no. 92194-027) states that "it was not expected that storage at -21°C would affect results negatively." Storage stability data for the longest period between sampling and extraction and analysis are required.

2. The 53-day half-life reported for vinclozolin in the Neuhofen soil (20 ± 2°C) is the same as that reported for another aerobic soil metabolism study reviewed separately (MRID no. 136376).

3. At time zero there was no TLC analysis hence no ability to detect positively parent and degradates. The only apparent analysis at time zero was for total radioactivity and radioactivity in methanol extracts.

4. The study lacks clear data tables which can easily be related to TLC data which show percent of radioactivity detected at Rf values. It also lacks a table which clearly identifies all degradates detected and the concentrations in which they were found and sample calculations which tie TLC results to data tables.

5. Raw data and figures which follow the study narrative are difficult to interpret because they are not labeled clearly and because the headings are in German. However, a key to the German headings was included in MRID no. 136376.

6. There was no confirmatory analytical method used for positive identification of compounds. EFGWB prefers that ^{14}C residues in samples be separated by chromatographic methods with at least three solvent systems of different polarity, and that the identity of specific compounds isolated by chromatography be verified using a confirmatory method such as GC/MS in addition to comparison to the R_f of reference standards.

7. The study was conducted with a German soil. Refer to p. 9 (copy attached) of the study summary for soil characteristics.

   a. The study and summary report that the soil is classified as a loamy sand in the German system and as a sandy loam according to the USDA soil classification system. It is difficult to confirm the textural classification according to the USDA system because the study summary (MRID no. 92194-027) reports soil particle size distribution according to the German system. The German and USDA systems are not directly comparable because the sand fraction (0.02-2mm) in the German system overlaps with the silt fraction (0.002-0.05mm) in the USDA system. The particle size breakdown should be reported according to the USDA system.

   b. Organic carbon, not organic matter, is reported. It is generally agreed that percent organic matter can be estimated by percent organic carbon multiplied by 1.724. Percent organic matter should be measured and reported.

   c. Cation exchange capacity (CEC) is reported as mVal/100g, which is identical to milliequivalents/100g (meq/100g). CEC should be reported as meq/100g or cmol(+)/kg.

   d. The registrant submitted a study which compares some

characteristics of the German Neuhofen soil with 12 U.S. soils (MRID no. 414969-03). Copies of Tables 1-4 from this study are attached.

With the exception of organic carbon and humus content, the chemical and physical characteristics of the Neuhofen soil fall within the range of values reported for the 12 U.S. soils (Table 1). The Neuhofen soil was higher in humus and organic carbon content (4.1 and 2.44%, respectively) than the U.S. soils to which it was compared. The U.S. soils had humus contents ranging from 1.1-2.2% and organic carbon contents ranging from 0.64-1.28%.

EFGWB notes that for the U.S. soils, organic carbon content was multiplied by 1.724 to obtain the percent of organic matter. Apparently the organic matter content of the Neuhofen soil was measured and not converted by multiplication.

Analyses of variance for the soils were performed for comparisons of total bacteria, actinomycetes, and fungi. In many cases significant differences were detected among the U.S. soils for these parameters. Significant differences also were found between the Neuhofen and some U.S. soils. However, the Neuhofen soil was within the ranges of values reported for U.S. soils for total bacteria, actinomycetes, and fungi; ratio of bacteria: actinomycetes: fungi; total Gram-negative bacteria; and percent of total bacteria comprised by Gram-negative species.

The Neuhofen soil has chemical, physical, and biological characteristics comparable to the U.S. soils studied. Therefore, EFGWB concludes that the Neuhofen soil is acceptable for conducting an aerobic soil metabolism study for vinclozolin.

8. The study reports a vinclozolin half-life of approximately 45 days which apparently was estimated from a residue decline curve. The study summary (MRID no. 92194-027) reports a half-life of 53 days calculated by regression analysis. EFGWB agrees with the 53-day half-life.

9. The soil water content was reported as 40% of maximum water-holding capacity. The study summary reports that this water content is comparable to 75% of 1/3 bar, the soil water content preferred by EFGWB for aerobic soil metabolism studies. There are no data submitted which confirm that the two moisture contents are comparable. Data which demonstrate the relative equality of these two soil water measurements are required.

10. The number of samples taken at each interval was not reported.

11. Material balances as calculated by the reviewer were 78-110%. For each sampling time, methanol-extractable and non-methanol extractable radioactivity were summed and compared with the total ^{14}C residues reported (Table 1).

12. Volatiles were not trapped, but $14CO_2$ was measured during the study. The total ^{14}C measured at each sampling time indicates that there was no

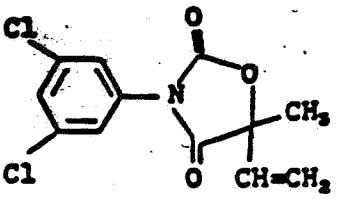
apparent loss of vinclozolin through volatilization.

13. The study summary indicates that metabolite B and vinclozolin exist in a pH-dependent equilibrium. This conclusion was based on a hydrolysis study which will be reviewed in Phase V.

14. According to the study summary, the soil was held in a climatized room where "the temperature range was more likely 20 ± 2°C." EFGWB prefers that aerobic soil metabolism studies be conducted at 18-30 ± 1°C.

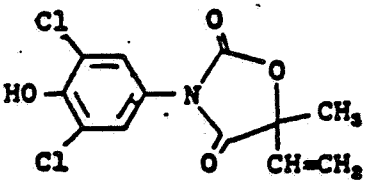
15. $^{14}CO_2$ evolution was reported as slight (0.53% of total radioactivity was found as radio-labeled CO_2 after 45 days) and remained constant. The reviewer could not confirm this independently. However, material balances and total radioactive residues measured at each sampling time tend to confirm that very little radioactivity was lost to $^{14}CO_2$ evolution.

FIGURE III



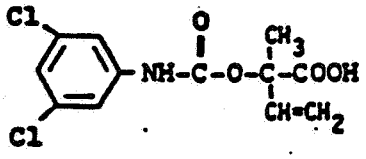
BAS 352 F, vinclozolin

3-(3.5-dichlorophenyl)-5-ethenyl-5-methyl-1.3-oxazolidine-2.4-dione



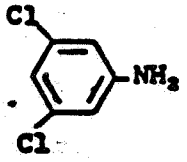
A

3-(3.5-dichloro-4-hydroxyphenyl)-5-ethenyl-5-methyl-1.3-oxazolidine-2.4-dione



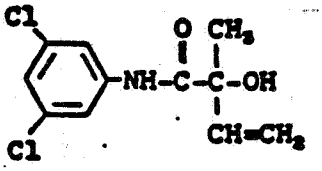
B

3.5-dichlorophenyl-carbamic acid(1-carboxy-1-methyl)-2-propenyl ester



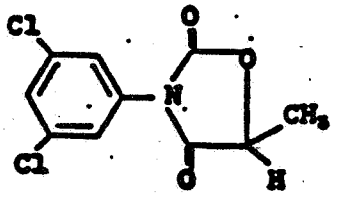
D

3.5-dichloroaniline



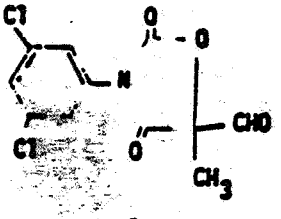
E

N-(3.5-dichlorophenyl)-2-hydroxy-2-methyl-3-butenoic acid amide



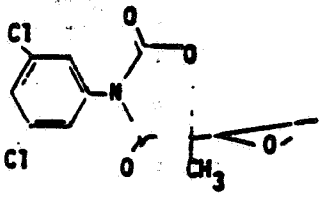
S

3-(3.5-dichlorophenyl)-5-methyl-1.3-oxazolidine-2.4-dione



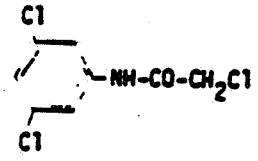
U

3-(3.5-dichlorophenyl)-5-formyl-5-methyl-1.3-oxazolidine-2.4-dione



V

3-(3.5-dichlorophenyl)-5-oxiranyl-5-methyl-1.3-oxazolidine-2.4-dione



DCAD

N-(3.5-dichlorophenyl)-chloro-acetamide

VINCLOZALIN Sh# 113201

Page_____ is not included in this copy.

Pages 51 through 57 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

3/25/91

DATA EVALUATION RECORD

STUDY IDENTIFICATION:

Huber, R. and S. Otto. 1978. Further investigations into the aerobic soil metabolism of BAS 352 F-^{14}C (^{14}C-vinclozolin). MRID no. 136376. BASF Lab Report No. 1571. (The study is summarized in MRID no. 92194-027.)

Reviewer's note: This DER should be reviewed in conjunction with DERs for two other aerobic soil metabolism studies (MRID nos. 88288 and 136377)

REVIEWED BY:

Arnet W. Jones, Agronomist
Review Section I, EFGWB

Signature: Arnet W. Jones

Date: MAR 25 1991

APPROVED BY:

Paul J. Mastradone, Ph.D., Chief
Review Section I, EFGWB

Signature: Paul J. Mastradone

Date: MAR 25 1991

TYPE OF STUDY: Aerobic Soil Metabolism (161-2)

CONCLUSIONS:

1. The study provides supplemental information regarding the aerobic soil metabolism of vinclozolin.

2. In three aerobic soil metabolism experiments carried out for 120 days at 20 ± 2°C or 25 ± 2°C using a Neuhofen (German) soil judged to be comparable to U.S. soils, radiolabeled vinclozolin degraded with half-lives 34.7 and 40.8 days (25 ± 2°C) and 53.3 days (20 ± 2°C). Vinclozolin degradates identified as metabolites S, E, D, and B were detected (see structures attached).

3. In two experiments conducted in a Pfungstadt (German) soil, radiolabeled vinclozolin underwent biphasic degradation. During the first 14 days, vinclozolin half-lives were 6.9 and 5.4 days for experiments carried out at 20 and 25°C, respectively. From day 14 until the end of the experiment (day 120), the rate of degradation slowed. Half-lives for this period were 46.2 and 49.5 days for the two respective temperatures. Vinclozolin degradates identified as metabolites S, E, D, and B were detected at each sampling time.

4. The data requirement for aerobic soil metabolism (161-2) will be reviewed for acceptance when frozen storage stability data for the longest period between sampling and extraction and analysis are submitted for the experiments conducted in the Neuhofen soil. The registrant also should confirm that there were no unknown degradation products formed in concentrations ≥0.01 ppm.

5. The studies conducted with the Pfungstadt soil will be considered supplemental because:

a) no data were presented which demonstrate that the soil compares with U.S. soils; and

b) frozen storage stability data for the longest period between sampling and extraction and analysis were not presented.

MATERIALS AND METHODS:

Test Substances

Vinclozolin (BAS 352 F), labeled in two different positions, was used in separate experiments. The test chemicals were phenyl-labeled vinclozolin (purity >98%; specific activity 9.23 mCi/mmol) and vinclozolin labeled in position 5 (referred to as 5-^{14}C throughout this DER) of the heterocyclic ring (purity >98%; specific activity 3.04 mCi/mmol). Non-labeled reference compounds also were used (see structures attached).

Soils

Two German soils, Neuhofen loamy sand and Pfungstadt loam, were used. Soil textures were determined according to the German system (see Discussion). The Neuhofen soil had the following characteristics: pH - 6.8; CEC - 10 mVal/100g; and organic carbon - 2.6%. The Pfungstadt soil had the following characteristics: pH - 7.4; CEC - 13 mVal/100g; and organic carbon - 0.7%. The moisture content of the Neuhofen and Pfungstadt soils was maintained at 15.7 and 13.3%, respectively, (40% of maximum water holding capacity for each soil) by weighing weekly and adding distilled water as necessary.

Experiments were carried out using the following combinations of radiolabeled test chemical, temperature, and soil:

- Neuhofen soil, phenyl-labeled vinclozolin, temp. 25 ± 2°C
- Neuhofen soil, 5-^{14}C-labeled vinclozolin, temp. 20 ± 2°C
- Neuhofen soil, 5-^{14}C-labeled vinclozolin, temp. 25 ± 2°C
- Pfungstadt soil, phenyl-labeled vinclozolin, temp. 20 ± 2°C
- Pfungstadt soil, phenyl-labeled vinclozolin, temp. 25 ± 2°C

Sampling

In each of the five experiments, 2 kg of soil was fortified to reach a vinclozolin concentration of 7 ppm. At 0, 7, 14, 30, 60, 90, and 120 days, a 100 g soil sample was taken and stored in a closed bottle at -21°C until analyzed.

Analysis

An aliquot of soil (200-800 mg) was combusted in duplicate to determine total radioactivity. Additional soil was extracted with methanol. Methanol extracts were analyzed by LSC; soil also was combusted to determine the non-methanol extractable radioactivity. Methanol extracts were partitioned with ethyl acetate and water. The ethyl acetate extracts were dried over Na_2SO_4 and brought to volume.

Aliquots of the ethyl acetate phase were lined onto silica gel TLC plates and developed separately in two solvent systems (chloroform and chloroform/acetic acid 95:5 [v/v]). Non-radioactive reference compounds (vinclozolin plus metabolites A, B, D, E, and S) were co-chromatographed on each plate. Following development and drying, the plates were scanned by TLC scanner to locate radioactive spots. Also, TLC plates were exposed to X-ray film for at least 3 days.

The ethyl acetate phase was evaporated almost to dryness and passed into a column containing 10 g of silica gel. Two eluents were used: (1) n-hexane/i-propanol 95:5 [v:v]; and (2) n-hexane/i-propanol/H_2O/formic acid 900:100:2:0.5 [v/v/v/v]. Aliquots from each eluent were analyzed by LSC.

Both eluents were evaporated to dryness and dissolved in 1 mL of eluent 2. HPLC of both eluates was performed with a UV detector (254 nm) in series with a radioactivity detector.

The identity of metabolites originally identified by HPLC retention times was confirmed by GC/MS. Spectra obtained were compared to reference standards.

REPORTED RESULTS:

General Results

Vinclozolin half-lives were reported to be 1.5-4 days in the loam soil (Pfungstadt soil - pH 7.4) and 23-42 days in the loamy sand (Neuhofen soil - pH 6.8). These half-lives were estimated from residue decline curves by assessing the time required for the initial vinclozolin concentration to decline by one-half. Generally, vinclozolin degraded more rapidly at 25°C than at 20°C. Also, the residue decline rate was reported to be related to soil pH, with faster degradation observed at pH 7.4 than at 6.8. Also, metabolite B was formed in greater quantities in the Pfungstadt soil (pH 7.4) than in the Neuhofen soil (pH 6.8) - see Tables III, VII, and XI.

The results of the individual experiments are presented below.

Neuhofen soil; phenyl-labeled vinclozolin; 25 +/- 2°C (Table III)

The half-life of vinclozolin under these conditions was not reported in the study nor in the summary. The reviewer-calculated half-life is 40.8

days.

As measured by HPLC, vinclozolin residues declined from 7.10 ppm at day 0 to 0.95 ppm at 120 days. Metabolites S, E, D, and B were detected at each sampling time in the amounts shown in Table III. Total radioactivity, expressed as vinclozolin ppm equivalents, was relatively constant and ranged between 7.68 ppm (day 0) and 7.41 ppm (day 60).

Material balances were not reported in the study but were reported in the summary (MRID no. 92194-027) as ranging between ranged between 96.7-108.4%. These material balances were calculated against an assumed initial vinclozolin concentration of 7.00 ppm, not against a measured quantity of radioactivity. Reviewer-calculated material balances were 88.5-100.8% (see Discussion).

Neuhofen soil, 5-^{14}C-labeled vinclozolin, temp. 20 +/- 2°C (Table VII)

The half-life of vinclozolin in this experiment was reported in the study summary as 53 days. The summary also reported a combined half-life of 60 days for vinclozolin plus metabolite B (p. 15 of summary - attached).

As measured by HPLC, vinclozolin residues declined from 6.73 ppm at day 0 to 1.39 ppm at day 120. Metabolites B, S, and E also were detected as indicated in Table VII. Metabolite D (3,5 dichloroaniline) was not detected in this experiment.

Material balances for this experiment were not reported in the study nor in the summary.

Neuhofen soil, 5-^{14}C-labeled vinclozolin, temp. 25 +/- 2°C (Table VII)

The half-life of vinclozolin in this experiment was not reported. The reviewer-calculated half-life is 34.7 days.

As measured by HPLC, vinclozolin residues declined from 6.68 ppm at day 0 to 0.61 ppm at day 120. Metabolites B, S, and E also were detected as indicated in Table VII. Metabolite D (3,5 dichloroaniline) was not detected in this experiment.

Material balances for this experiment were not reported in the study nor in the summary.

Pfungstadt soil, phenyl-labeled vinclozolin, temp. 20 +/- 2°C (Table XI)

The study discussion indicates that the half-life of vinclozolin in the Pfungstadt soil ranged between 1.5-4 days. As measured by HPLC, vinclozolin residues declined from 6.37 ppm at day 0 to 0.33 ppm at 120 days. Metabolites S, E, D, and B were detected at each sampling time in the amounts shown in Table XI.

The degradation of vinclozolin in the Pfungstadt soil appears to be

biphasic, i.e., residues declined rapidly during the first part of the experiment, with the rate of degradation tapering off as the initial concentration of vinclozolin declined. From an initial vinclozolin concentration of 6.37 ppm at day 0, the concentration declined to 1.60 ppm by day 14. This yields a half-life (reviewer-calculated) of 6.9 days for the first two weeks of the experiment. For the remainder of the study, the degradation proceeded more slowly with the half-life being 46.2 days (reviewer-calculated) for the last 106 days of the experiment.

Total radioactivity, expressed as vinclozolin ppm equivalents, was relatively constant and ranged between 6.52 ppm (day 30) and 7.08 ppm (day 14).

Material balances were not reported in the study but were reported in the summary (MRID no. 92194-027) as ranging between ranged between 96.7-108.4% (p. 13 of summary - attached). These material balances were calculated against an assumed initial vinclozolin concentration of 7.00 ppm, not against a measured quantity of radioactivity. The reviewer-calculated material balances were 94.9-108.7% (see Discussion).

Pfungstadt soil, phenyl-labeled vinclozolin, temp. 25 + 2°C (Table XI)

The study discussion indicates that the half-life of vinclozolin in the Pfungstadt soil ranged between 1.5-4 days. As measured by HPLC, vinclozolin residues declined from 6.03 ppm at day 0 to 0.23 ppm at 120 days. Metabolites S, E, D, and B were detected at each sampling time in the amounts shown in Table XI.

The degradation of vinclozolin in the Pfungstadt soil appears to be biphasic, i.e., residues declined rapidly during the first part of the experiment, with the rate of degradation tapering off as the initial concentration of vinclozolin declined. From an initial vinclozolin concentration of 6.03 ppm at day 0, the concentration declined to 1.01 ppm by day 14. This yields a half-life (reviewer-calculated) of 5.4 days for the first two weeks of the experiment. For the remainder of the study, the degradation proceeded more slowly with the half-life being 49.5 days (reviewer-calculated) between days 14 and 120.

Total radioactivity, expressed as vinclozolin ppm equivalents, was relatively constant and ranged between 6.42 ppm (day 120) and 6.98 ppm (day 30). The material balances were not given. The reviewer-calculated material balances were 81.2-104.8%.

DISCUSSION:

1. Storage stability data for the longest period between sampling and extraction and analysis are required. The study reports that soil samples were taken at sampling dates "and stored in closed flasks at -21°C until analyzed." The study summary (MRID no. 92194-027) states that "it was not expected that storage at -21°C would affect results negatively." This assumption is not supported by data. The time

interval between sampling and extraction and analysis must be specified and data must be presented which proves that vinclozolin and its metabolites remain stable in the frozen storage conditions used in these experiments.

2. Five different experiments using two soils, two temperatures, and two radiolabel positions, were conducted. Rather than report half-lives for each study, the discussion states that half-lives were 1.5-4 days in the loam (Pfungstadt) soil and 23-42 days in the loamy sand (Neuhofen) soil. These half-lives were estimated from the time required for the initial vinclozolin concentration to decline by one-half.

   In the Neuhofen soil, the degradation rate was relatively constant within a given experiment with degradation proceeding more quickly at 25°C than at 20°C. The longest half-life, 53.3 days, was observed in the experiment where the temperature was 20°C. In the experiments performed at 25°C, the half-lives were 34.7 and 40.8 days.

   In the Pfungstadt soil, the decline of vinclozolin residues appears to be biphasic with two relatively distinct rates of degradation. In two experiments conducted in this soil, vinclozolin degradation proceeded rapidly during the first 14 days with half-lives being 6.9 and 5.4 days for experiments carried out at 20 and 25 C, respectively. As initial vinclozolin levels declined during the first two weeks of the experiments, the rate of degradation slowed between day 14 to day 120. From day 14 to day 120, half-lives of 46.2 and 49.5 days were calculated by the reviewer for the respective temperatures.

3. There apparently were no unknown metabolites detected in quantities $\geq$0.01 ppm, but the study does make this clear. The registrant should confirm this.

4. The 53-day half-life reported for the Neuhofen soil (20 $\pm$ 2°C) is the same as that reported for another aerobic soil metabolism study reviewed separately (MRID no. 88288).

5. Sampling was not carried out at sufficiently short intervals in the Pfungstadt soil experiments where initial vinclozolin degradation was rapid (see item 2 above). Samples were taken at days 0, 7, and 14; sampling should have been done at shorter intervals, perhaps as often as daily, in order to allow a more accurate determination of the half-life.

6. Material balances were not reported in the study. But the summary (MRID no. 92194-027) reports a material balance for one of the five experiments (Neuhofen soil; phenyl-labeled vinclozolin; temperature held at 25 $\pm$ 2°C.) The material balance reported was not calculated from measured radioactivity but rather from an assumed initial vinclozolin concentration of 7.00 ppm. The reviewer-calculated material balances for these studies ranged between and 81.2 - 108.7%. Material balances for each sampling time were calculated by adding unextractable radioactivity to the sum of all residues detected (vinclozolin plus metabolites S, E, D, and B). This amount was then divided by the total

radioactivity measured at each sampling interval.

Material balances could not be calculated for experiments where vinclozolin was labeled in the heterocyclic ring. In these experiments there apparently was degradation of the heterocyclic ring and ^{14}C was lost to volatile degradation products. If ^{14}C-labeled volatiles had been trapped, material balances for all studies could have been calculated. Volatiles should be trapped to allow calculation of material balances.

7. The textures of test soils, reported in the German system, do not compare precisely with USDA system. The German and USDA systems are not directly comparable because the sand fraction (0.02-2mm) in the German system overlaps with the silt fraction (0.002-0.05mm) in the USDA system. The particle size breakdown should be reported according to the USDA system.

8. EFGWB requires that foreign soils used for environmental fate studies be comparable with U.S. soils. In a review of another aerobic soil metabolism study submitted for vinclozolin (MRID no. 88288), EFGWB concluded that the Neuhofen soil compares favorably with U.S. soils in physical and chemical characteristics. There were no data submitted for the Pfungstadt soil which would enable its comparison to U.S. soils Hence the data on the Pfungstadt soil will be considered supplemental.

9. According to the study summary, the soils were held in climatized rooms where "the temperature range was more likely 20 $\pm$ 2°C [or 25 $\pm$ 2°C]." EFGWB prefers that aerobic soil metabolism studies be conducted at 18-30 $\pm$ 1°C.

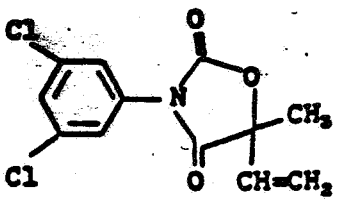
10 The study indicates that "the single application rate is 1 lb/A which at 4 inches depth would result in a concentration of 0.75 ppm in soil. A concentration of 7 ppm would include multiple and practical treatment pattern." According to the LUIS report of Nov. 6, 1990, the maximum amount of vinclozolin applied is on strawberry where six 2-pint applications of the flowable concentrate formulation can be made. EFGWB calculates that this use schedule would result in a total of 6.26 lb a.i./A, or a total vinclozolin concentration of approximately 5 ppm in the top 4 inches of soil (12 pints = 1.50 gal; density = 4.17 lb a.i./gal; 1.50 gal x 4.17 lb a.i./gal = 6.26 lb a.i.; 6.26 lb a.i./1.34 x 10^6 lb of soil = 4.67 ppm). However, this does not account for degradation of vinclozolin between the individual applications. Assuming proper usage according to label instructions, it is not likely that vinclozolin soil concentrations would reach 7 ppm in the top 4 inches. Terrestrial field dissipation studies should address the issue of vinclozolin accumulation under field conditions.

11. Metabolite D (3,5 dichloroaniline) was not detected in the studies where the radiolabel was placed in the heterocyclic ring (see structures of vinclozolin and metabolites attached). Although undetected, it is likely that 3,5 dichloroaniline was formed.

Metabolite D forms after degradation of the heterocyclic ring moiety of the vinclozolin molecule. Since metabolites apparently were quantified only from radio-HPLC data, it is logical that 3,5 dichloroaniline would not be detected because it would not contain ^{14}C which was only in the heterocyclic ring moiety.

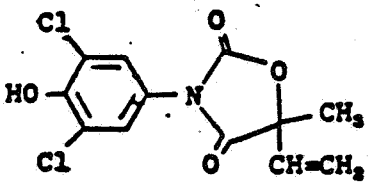
12. This study was originally reviewed in August 1979. A copy of the review is attached. Because it lacks detail, it could not be determined from the 1979 review whether the study satisfies the current data requirement.

FIGURE III



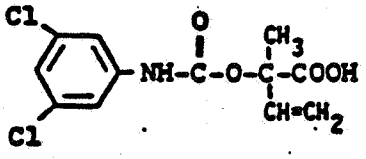
BAS 352 F, vinclozolin

3-(3.5-dichlorophenyl)-5-ethenyl-5-methyl-1.3-oxazolidine-2.4-dione



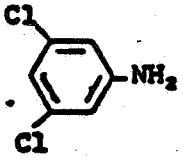
A

3-(3.5-dichloro-4-hydroxyphenyl)-5-ethenyl-5-methyl-1.3-oxazolidine-2.4-dione



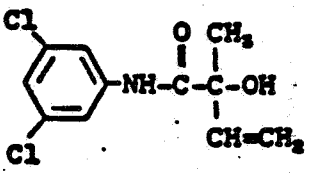
B

3.5-dichlorophenyl-carbamic acid(1-carboxy-1-methyl)-2-propenyl ester



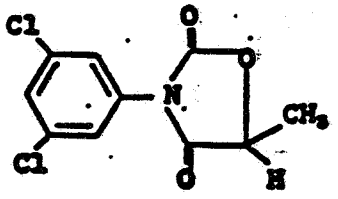
D

3.5-dichloroaniline



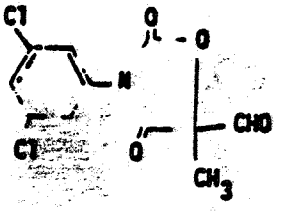
E

N-(3.5-dichlorophenyl)-2-hydroxy-2-methyl-3-butenoic acid amide



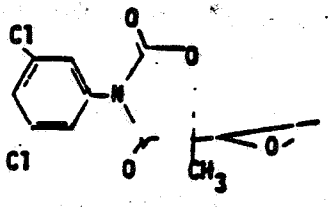
S

3-(3.5-dichlorophenyl)-5-methyl-1.3-oxazolidine-2.4-dione



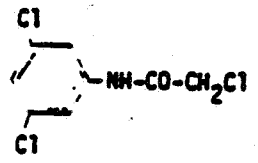
U

3-(3.5-dichlorophenyl)-5-formyl-5-methyl-1.3-oxazolidine-2.4-dione



V

3-(3.5-dichlorophenyl)-5-oxiranyl-5-methyl-1.3-oxazolidine-2.4-dione



DCAD

N-(3.5-dichlorophenyl)-chloro-acetamide

9

66

VINCLOZALIN Sh# 113201

Page_____ is not included in this copy.

Pages 67 through 71 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

CHEMICAL: Ronilan, Vinclozolin, 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione (formerly BAS 353F, BAS 352(04)F, 83 258)

FORMULATION: Technical

CITATION: Huber, R., S.Otto. (1978) Further Investigations into the Aerobic Soil Metabolism of BAS 352 F-^{14}C (^{14}C-Vinclozolin) (unpublished report prepared by BASF Wyandotte Corp., Parsippany, NJ, Laboratory Report No. 1571)

TRADE SECRET CLAIM: Yes

REASON FOR REVIEW: Registration of Ronilan

REVIEWED BY: W.S. Chou, Staff Scientist, Enviro Control, Inc. Rockville, MD

DATE OF REVIEW: August 24, 1979

TEST TYPE: Metabolism study

CONCLUSIONS:

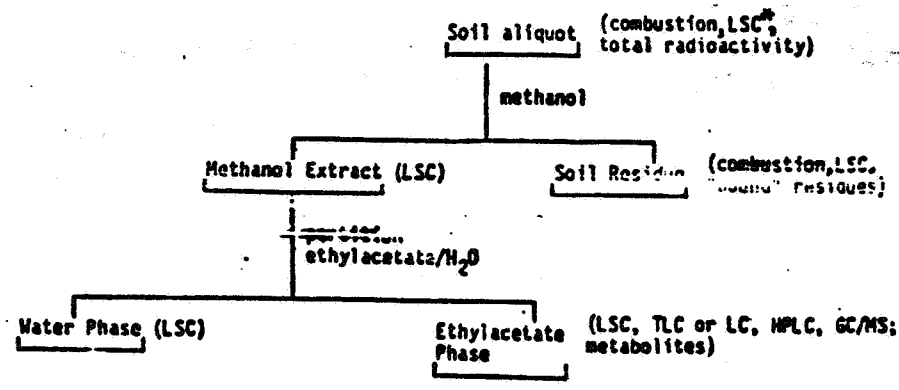
1. According to the study, BAS 352 F was not persistent in soil, and degraded faster in loam than in loamy sand.

2. Compounds B, E, S and D were identified as metabolites. Material balances, including nonextractable residue, were provided.

3. In the reviewer's opinion, the investigation was thoroughly performed and reported.

MATERIALS AND METHODS:

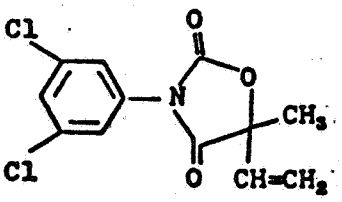
Aerobic soil metabolism of BAS-352 F was studied with two soils (loamy sand, loam), at two temperatures (20°C and 25°C) and with two differently labeled preparations of the compound, one labeled in its benzene ring and one labeled at carbon 5. 2kg of soil were fortified with 7 ppm labeled BAS 352F. Soil samples (100 gram) were taken from each experiement at 0, 7, 14, 30, 60, 90 and 120 days after the study started. Soil samples were fractionated as follows:



Each phase was radioassayed. Insoluble samples (soil) were combusted and counted with a liquid scintillation counter (LSC).

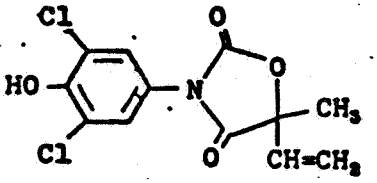
The following cold standards were available for comparison and were synthesized by BASF AG, GPE/RR, Limburgerhof (FRG), Dr. Ohmsorge.

Purity ⩾ 99 % ; structures were confirmed by MS, NMR, IR and elemental analyses.



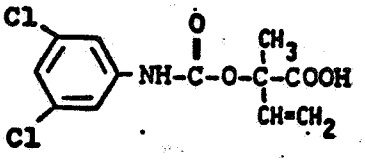
BAS 352 F

3-(3.5-dichlorophenyl)-5-ethenyl-5-methyl-1.3-oxazolidine-2.4-dione



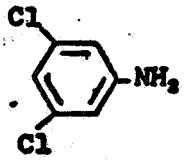
A

3-(3.5-dichloro-4-hydroxyphenyl)-5-ethenyl-5-methyl-1.3-oxazolidine-2.4-dione



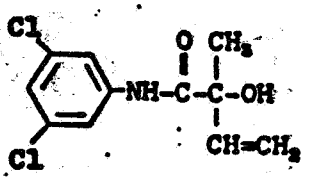
B

3.5-dichlorophenyl-carbamic acid(1-carboxy-1-methyl)-2-propenyl ester



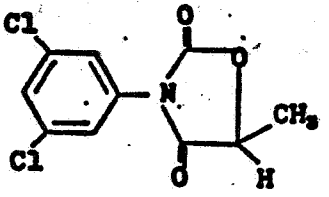
D

3.5-dichloroaniline



E

N-(3.5-dichlorophenyl)-2-hydroxy-2-methyl-3-butenoic acid amide



S

3-(3.5-dichlorophenyl)-5-methyl-1.3-oxazolidine-2.4-dione

For confirmation of metabolites, the ethyl acetate extract was subjected to TLC, LC, HPLC and MS analyses. The TLC plates were developed in two solvent systems. Radioactive metabolites were located with a TLC scanner. The concentrated ethyl acetate extract was dissolved in n-hexane/i-propanol and passed into an LC column using n-hexane/i-propanol for fraction I and n-hexane/i-propanol/H_2O/HCOOH for fraction II. HPLC of both fractions from LC was performed using a UV detector at 254 nm in series with a radioactivity detector to identify metabolites qualitatively and quantitatively. For MS-confirmation of metabolites, corresponding HPLC fractions from several runs were isolated, combined, concentrated and submitted to GC/MS. Spectra obtained were compared with reference standards.

REPORTED RESULTS:

1. The results (material balance) from radioactivity measurements of all experiments were shown in Tables III, VII, XI and graphically presented in Figures I-V.

2. By HPLC comparison with standards, parent compound BAS 352 F, metabolites J, E and D were located in fraction I, metabolite B was found exclusively in fraction II. The same result was found in all experiements. With the label in 5-position, metabolite D was not detectable by radioactivity measurements.

3. The half-life of BAS 352 F was rapid (1.5 - 4 days) in loam and about 10 times slower (23 - 42 days) in loamy sand. The total radioactivity remained constant over the experimental period when ring-labeled BAS 352 F was used, while the total radioactivity decreased with time when BAS 352 F labeled in the 5 position was used.

4. Methanol-extractable radioactivity decreased, whereas the bound radioactivity generally increased with time.

DISCUSSION:

1. Investigators suggested that the difference in half-life of BAS 352 F in loam and loamy sand was due to the instability of BAS 352 F to hydrolysis at higher pH values.

2. The degradation rate of BAS 352 F was increased as temperature increased.

3. The author suggested that the total radioactivity of the BAS 352 F labeled in the 5 position declined with time due to a partial destruction of the heterocyclic ring.

Table III: Balance of BAS 352 F-(phenyl-U-^{14}C) and metabolites in Neuhofen soil (loamy sand) and various fractions; Temp. 25 ± 2 °C - V 20/77

days	total radio-activity	Methanol-Extr.		Ethylacetate/H_2O partition		Calculated values after HPLC				
		Extr.	Soil Residue	Ethyl-acetate phase	H_2O phase	BAS 352 F	S	E	D	B
	WT 2	WT 1	WT 3	WT 4						
0	7.68	7.34	0.08	7.47	0.01	7.10	0.23	0.04	0.01	0.03
7	7.50	6.73	0.62	6.95	0.02	6.07	0.18	0.13	0.05	0.51
14	7.60	6.37	1.12	6.44	0.04	5.25	0.17	0.14	0.15	0.73
60	7.41	3.74	3.62	3.75	0.05	2.22	0.11	0.06	0.42	0.94
90	7.56	2.65	4.27	2.69	0.07	1.45	0.10	0.06	0.70	0.37
120	7.65	1.94	4.71	2.06	0.04	0.95	0.11	0.05	0.49	0.46

All values in ppm of BAS 352 F equivalents

WT referes to raw data sheet in attachment III.

DATA EVALUATION RECORD

STUDY IDENTIFICATION:

Hamm, R. 1978. Degradation of ^{14}C-vinclozolin (BAS 352 F) in soil under aerobic, anaerobic, and sterile conditions. MRID no. 136377. BASF Lab Report No. 1592. (The study is summarized in MRID no. 92194-027.)

Reviewer's Notes:

1. This DER should be reviewed in conjunction with DERs for two other aerobic soil metabolism studies (MRID nos. 88288 and 136376).

2. This DER is a supplement to the original review which was written in August 1979, a copy of which is attached. Refer to the original review for additional details regarding materials and methods, results, and discussion.

REVIEWED BY:

Arnet W. Jones, Agronomist
Review Section I, EFGWB

Signature: Arnet W. Jones

Date: MAR 25 1991

APPROVED BY:

Paul J. Mastradone, Ph.D., Chief
Review Section I, EFGWB

Signature: Paul J. Mastradone

Date: MAR 25 1991

TYPE OF STUDY: Aerobic Soil Metabolism (161-2)

CONCLUSIONS:

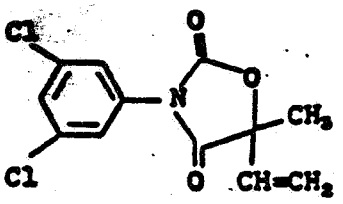
1. The study provides supplemental information regarding the aerobic soil metabolism of vinclozolin.

2. In a Neuhofen sandy loam soil at 20 ± 1°C, vinclozolin degraded under aerobic, anaerobic, and aerobic sterile conditions. Degradation proceeded at the fastest rate under aerobic conditions. Degradation products identified as metabolites B, D, and E were detected by TLC (see structures attached).

3. The study will be considered supplemental because degradation rates and the percent of radioactivity in various metabolites cannot be determined from the data.

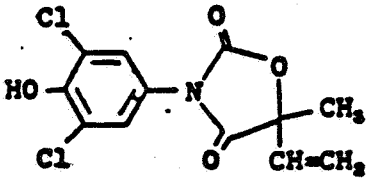
4. Refer to the original review of this study for additional details.

FIGURE III



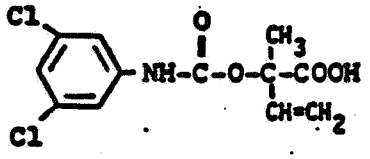
BAS 352 F, vinclozolin

3-(3.5-dichlorophenyl)-5-ethenyl-5-methyl-1.3-oxazolidine-2.4-dione



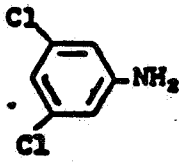
A

3-(3.5-dichloro-4-hydroxyphenyl)-5-ethenyl-5-methyl-1.3-oxazolidine-2.4-dione



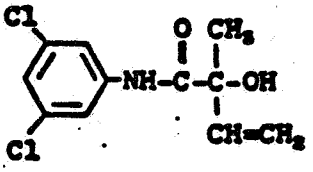
B

3.5-dichlorophenyl-carbamic acid(1-carboxy-1-methyl)2-propenyl ester



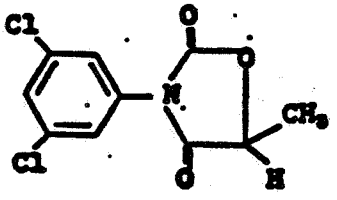
D

3.5-dichloroaniline



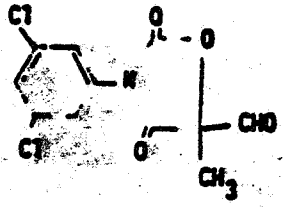
E

N-(3.5-dichlorophenyl)-2-hydroxy-2-methyl-3-butenoic acid amide



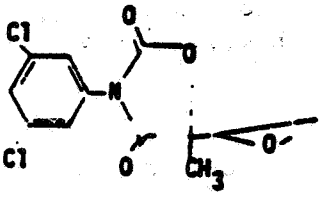
S

3-(3.5-dichlorophenyl)-5-methyl-1.3-oxazolidine-2.4-dione



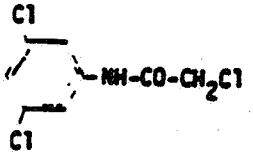
U

3-(3.5-dichlorophenyl)-5-formyl-5-methyl-1.3-oxazolidine-2.4-dione



V

3-(3.5-dichlorophenyl)-5-oxiranyl-5-methyl-1.3-oxazolidine-2.4-dione



DCAD

N-(3.5-dichlorophenyl)-chloro-acetamide

77

CHEMICAL: Ronilan, Vinclozolin, 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione (formerly BAS 353 F, BAS 352(04)F, 83 258)

FORMULATION: Technical

CITATION: Hamm, R. (1978). Degradation of 14C-Vinclozolin (BAS 352 F) in Soil under Aerobic, Anaerobic and Sterile Conditions (unpublished report prepared by BASF Wyandotte Corp., Parsippany, NJ, Report No. 1592)

TRADE SECRET CLAIM: Yes

REASON FOR REVIEW: Registration of Ronilan

REVIEWED BY: W. S. Chou, Staff Scientist, Envirq Control, Inc. Rockville, MD

DATE OF REVIEW: August 23, 1979

TEST TYPE: Soil Metabolism Study

CONCLUSIONS:

1. The study showed that the pesticide was degraded under all experimental conditions.

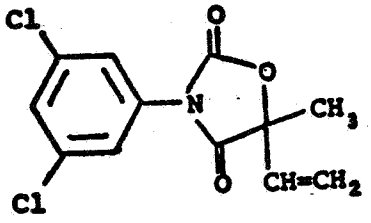
2. The largest degradation rate was obtained under aerobic, and the lowest under aerobic-sterile conditions

3. Three metabolites were identified.

4. The microorganism population was not affected by BAS 352 F at the experimental dose (2 mg pesticide added to 170 g soil).

5. The reviewer considers the study design appropriate for the data presented. However, degradation rates and the percent of radioactivity in various metabolites could not be determined from the data. This information would be necessary in order for the study to fully comply with EPA data requirements.

MATERIALS AND METHODS:



BAS 352 F - Vinclozolin
3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-1,3-oxazolidine-2,4-dione

A loamy sand was used. Labeled BAS 352F (specific activity 4 mCi/mMole) and standard substances for metabolite identification were synthesized.

B 78

Soil (170g) and 2 mg BAS 352 F were placed in a flask and moistened with water. The flask was then plugged with cotton, wrapped (light-proof) with aluminum foil and stored in a controlled climate chamber. For anaerobic studies, the soil was saturated with water and flushed with nitrogen for 2 hours. For the sterile aerobic series, the flask filled with soil was sterilized three times and pesticides were added on a lamirar flow bench.

Samples for aerobic and aerobic-sterile conditions were taken at 3, 7, 14, 21, 28 and 60 days. For the anaerobic series, samples were taken at 28 and 60 days.

Soil samples were extracted with methanol and filtered; 1 ml of the total extract (500 ml) was used for the determination of the extractable carbon-14 radioactivity. Radioactivity was measured with a scintillation counter.

Ten ml of the methanol extract was dried and dissolved in 0.5 ml methanol, 100 µl of this solution was applied to precoated TLC plates. After cnromatogram development the plates were a scanned with a TLC scanner.

Soil microorganism populations for the three experimental conditions were determined according to the Koch dish technique at various intervals.

REPORTED RESULTS:

1. The extractability of radioactive materials decreased in all experiments as the sampling interval increased, but was most rapid under aerobic condition.

2. Three day samples showed that the same degradation products could be detected by TLC in both aerobic and aerobic-sterile batches; however, the products appeared in far lower concentrations under sterile condition (Figure 1).

3. Variation in the relative concentrations of individual metabolites occured as the experiment progressed. The concentration of metabolite D decreased during the course of the study. At the end of the experiment, BAS 352 F was degraded under all three conditions to the known metabolites B, D, and E and other more polar substances (Figure 2).

4. The degradation rate was highest under aerobic and lowest under sterile-aerobic conditions. The degradation process was essentially the same under all three experimental conditions.

5. Comparisons of total organisms in selected species in untreated and treated soils (aerobic and anaerobic) showed no pesticide effects (Figures 3-6).

DISCUSSION:

1. The field application rate of BAS 352 F was not included in this study, therefore the reviewer has no base to evaluate whether the experimental dose rate was adequate or not.

2. If a non-polar solvent was used followed by a methanol extraction, the extractability of radioactive substances may increase considerably and more information on metabolites might be obtained at the end of the experiment.

3. Data on degradation of the pesticide as reported do not allow the reviewer to determine if a 90% loss of the pesticide occurred, as required in EPA guidelines.

4. In addition, the data do not allow the reviewer to determine which degradation products might have exceeded 10% of the original activity, and which would therefore require identification as per EPA guidelines.

VINCLOZALIN Sh# 113201

Page_____ is not included in this copy.

Pages 81 through 85 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DATA EVALUATION RECORD

STUDY IDENTIFICATION:

Study 1: Kuc, W.J. 1978. ^{14}C-BAS 352 F (vinclozolin) bluegill sunfish (Lepomis macrochirus) bioconcentration study. MRID no. 136387. Unpublished study prepared by Union Carbide Environmental Services for BASF Wyandotte Corp., UCES project no. 11506-80.

Study 2: Portnoy, C.E. and J.R. Clark. 1978. Identification of ^{14}C residues in water and fish from the BAS 352 F ^{14}C bluegill sunfish accumulation study. Unpublished report prepared by BASF Wyandotte Corp., Laboratory report no. PM-22. (Submitted as part of Study 1 - there is no separate MRID no.)

Reviewer's Note: This DER supplements the reviews of Aug. 30, 1979 (Study 1) and Aug. 24, 1979 (Study 2) by Enviro Control, Inc. Copies of these reviews are attached to this DER. Also, both studies are summarized in MRID no. 92194-031.

REVIEWED BY:

Arnet W. Jones, Agronomist
Review Section I, EFGWB

Signature: Arnet W. Jones

Date: MAR 20 1991

APPROVED BY:

Paul J. Mastradone, Ph.D.
Chief, Review Section I, EFGWB

Signature: Paul J. Mastradone

Date: MAR 25 1991

TYPE OF STUDY: Bioaccumulation in fish (165-4)

CONCLUSIONS:

General:

1. The studies partially fulfill the data requirement for accumulation of vinclozolin and its degradates in fish. Data on the uptake and depuration of vinclozolin by bluegill sunfish are acceptable.

2. The data requirement for bioaccumulation in fish will be reconsidered when the following issues are addressed:

   a) identification of residues characterized by TLC must be confirmed by a method such as GC/MS;

   b) all degradates detected in concentrations $\geq$ 0.01 ppm must be identified; and

   c) acceptable frozen storage stability data on vinclozolin and its

86

degradates in fish tissue must be presented.

If these data cannot be provided, a new study must be conducted.

Study 1:

1. The study is acceptable and partially fulfills the data requirement for vinclozolin accumulation in fish.

2. When exposed to 0.465 ± 0.047 ppm ring-labeled ^{14}C-vinclozolin in a flow-through experiment for 30 days, bluegill sunfish accumulated maximum ^{14}C residues of 50, 154, and 115 ppm in edible, non-edible, and whole fish tissue, respectively. The respective maximum bioconcentration factors were 106, 317, and 241. Maximum accumulation of radioactive residues occurred on day 14 of uptake.

3. After 14 days of depuration, ^{14}C residues declined by 96.9%, 97.1%, and 97.7% from maximum observed concentrations in edible, non-edible, and whole fish tissue, respectively.

Study 2:

1. The study provides supplemental information regarding identification of vinclozolin and its degradates in fish tissue.

2. In a study designed to determine the chemical identity of ^{14}C residues in water and bluegill sunfish during 30 days of exposure to 0.465 ppm of ring-labeled vinclozolin, the major radioactive compounds identified by TLC in water extracted with ethyl acetate were metabolite B, vinclozolin, and metabolite E. Vinclozolin, metabolite F, and two unknown metabolites were the primary ^{14}C-labeled compounds detected by TLC in edible and non-edible fish tissue.

3. Additional data are required regarding:

   a) confirmation of the identity of vinclozolin degradates in fish tissue;

   b) identification of degradates which occur at ≥ 0.01 ppm; and

   c) frozen storage stability data for vinclozolin and its degradates in fish tissue.

   Refer to Discussion for details.

MATERIALS AND METHODS:

Refer to the Enviro Control reviews of Aug. 24 and 30, 1979 (attached) for a general description of materials and methods. Additional information which does not appear in the previous reviews appears below:

87

Study 1:

1. During the uptake portion of the study, water and fish were sampled on days 1, 3, 7, 10, 14, 22, and 30. Water also was sampled on day 0. Fish and water were sampled on days 1, 3, 7, 10, and 14 of depuration.

2. The pH of the exposure water ranged between 7.70 and 8.06. In depuration water, pH ranged between 7.88 and 8.02. The water temperature in exposure and depuration tanks was 21.2 ± 0.9°C and 22.5 ± 0.8°C, respectively.

3. At each sampling date, 10 fish tissue samples were analyzed separately for total radioactivity. Also, the total counting data for all fish tissue of a specific type for a given sampling date were computed as a single sample to yield a weighted average. The weighted average was used to calculate the bioconcentration factors reported.

Study 2:

1. On days 7, 14, and 30 of the uptake phase, 40 fish were removed, dissected into edible and non-edible sections, and frozen for subsequent identification of ^{14}C residues.

REPORTED RESULTS:

Study 1:

Refer to Enviro Control review of Aug. 30, 1979 (attached) for a general outline of results for study 1.

1. ^{14}C residues exceeding background levels and the detection limit (0.08 ppm) were reported for control fish (whole fish tissue) on two sampling dates during the uptake phase (day 7 - 0.104 ppm; day 30 - 0.247 ppm). On day 3 of depuration, a ^{14}C residue of 0.165 ppm was measured in control fish. Radiocarbon residues exceeding background levels were not reported for control water. See Tables 3 and 4 (copies attached).

Study 2:

1. The major ^{14}C residues detected by TLC in ethyl acetate extracts of water were metabolite B, vinclozolin, and metabolite F (Table III). The respective ranges of concentrations for these compounds were 40.1-46.7%, 12.5-27.2%, and 9.3-10.6% of the total ^{14}C found in water. Of the total ^{14}C in water, 91.8-96.9% was extracted with ethyl acetate over the four sampling dates.

2. In fish tissue, only parent vinclozolin and metabolite F were identified by TLC (Table IV). Vinclozolin comprised 34.0-37.3% of the total ^{14}C detected in edible fish tissue and 51.7-61.9% in non-edible tissue. Metabolite F comprised 17.5-24.7% and 6.3-9.3% of the total

^{14}C in edible and non-edible tissue, respectively.

3. Two unknown compounds also were detected. One compound (unknown 1) comprised 9.5% of the total ^{14}C in edible tissue in day 7 samples, the largest amount of unknown radioactivity attributed to a single compound in a given tissue sample. In addition to the radioactivity attributed to the four compounds in Table IV, an additional 8-14% of the total ^{14}C in each sample was unidentifiable material.

DISCUSSION:

Refer to Enviro Control review of Aug. 30, 1979 (attached) for a general discussion of the study.

Study 1:

1. Detection limits of 0.08 and 0.009 ppm were reported for fish tissue and water, respectively.

2. Total mortality for the 44-day test period was reported as two fish, both from the control aquarium.

3. According to EEB records the vinclozolin 96-hr LC_{50} for bluegill sunfish is 47.3 ppm.

Study 2:

1. The identity of ^{14}C residues in samples separated by chromatographic methods should be verified using a confirmatory method such as GC/MS. In this study, radioactive areas on the TLC plates were identified by comparison to the location of known reference standards chromatographed on the same plates.

   It is not necessary that identity of degradates be confirmed in this manner for each environmental fate study. If it can be demonstrated in another acceptable environmental fate study that the identity of specific degradates characterized by chromatographic methods can be confirmed by a method such as GC/MS, chromatographic characterization of those same degradates will suffice for other studies provided that identical solvent systems are used.

2. The detection limit for vinclozolin and its degradates in fish tissue was not stated in the study. Apparently the detection limit is 0.08 ppm, the same as study 1. The analytical method used is not sufficiently sensitive to enable identification of unknown degradates which occur in concentrations as low as 0.01 ppm.

   Degradates in fish tissue which exceed 10% of applied radioactivity or 0.01 ppm, whichever is less, should be identified. Table IV (copy attached) indicates that one unidentified compound (unknown 1) comprised 9.5% of the total applied radioactivity in edible tissue at day 7 of the uptake phase of the study. This amounts to a

89

concentration of 0.04 ppm (the initial vinclozolin exposure concentration was 0.465 ppm). The identity of this unknown vinclozolin degradate should be determined. Further, Table IV indicates that "an additional 8-14% of the total ^{14}C in each sample was distributed over the three TLC plates used for each analysis as unidentifiable material." The nature of the unidentified material should be explained more completely.

3. The study summary (MRID no. 92194-031) indicates that fish tissue collected for residue identification was frozen (the temperature was not specified) "for less than two months" while awaiting analysis. As justification for not providing frozen storage stability data, the summary indicates that "studies have determined that vinclozolin is sufficiently stable in frozen animal matrices [eggs and tissue of laying hens; milk and tissue of dairy cows] for up to one year without affecting the validity of subsequent analytical measurements." A discussion with HED indicated that there has been no acceptance of storage stability data in these animal matrices. Accordingly, EFGWB will require acceptable storage stability data for vinclozolin and its degradates in fish tissue.

4. At days 7, 14, and 30 of uptake and at day 14 of depuration, 40 fish were removed for characterization of residues. Test fish were dissected into edible and non-edible parts and frozen (temperature unspecified) in glass vials. Apparently all edible and non-edible tissues were composited, the tissue was homogenized, and a single 5-6 g sample was analyzed for residue identification. This should be clarified.

CHEMICAL: Ronilan, Vinclozolin, 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione (formerly BAS 353F, BAS 352(04)F,83 258)

FORMULATION: Technical

CITATION: Kuc, W.J. (1978). ^{14}C-BAS 352 F Bluegill Sunfish (Lepomis macrochirus Bioconcentration Study (unpublished report prepared by Union Carbide Corp., Tarrytown, NY, for BASF Wyandotte Corp., Parsippany, NJ)

TRADE SECRET CLAIM: Yes

REASON for REVIEW: Registration of Ronilan

REVIEWED BY: S. Gould, Staff Scientist, Enviro Control, Inc. Rockville, MD

DATE of REVIEW: August 30, 1979

TEST TYPE: Fish Accumulation Study

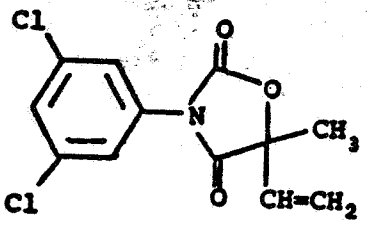
CONCLUSIONS:

1. Maximum residue concentration occurred on day 14 and was 115 ppm for whole fish, 50 ppm for edible fish, and 154 for nonedible tissue. Residue elimination after depuration was approximately 97% for whole fish, and edible and nonedible tissue.

2. The protocol was described in depth, and the study appeared to be well performed. This study met flow-through system requirements in EPA guidelines.

MATERIALS AND METHODS:

A flowthrough bluegill sunfish bioconcentration study was conducted using BAS 352 F labeled with carbon-14 in the benzene ring to provide a concentration of 0.50 ppm in the water.



BAS 352 F - Vinclozolin
3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-1,3-oxazolidine-2,4-dione

The test consisted of a 30-day uptake phase and a 14-day depuration period. Two-month-old bluegill sunfish were obtained from a commercial hatchery and were maintained for 4 months prior to testing. After random selection from the stock culture, 350 fish (mean weight 1.70 g) were added to each of two glass aquaria (1 test and 1 control) containing 158 l of softened well water. They were maintained for 24 hours before initiation of the period. The flow rate was 1.0 liter per 6-minute cycle during uptake and the first 7 days of depuration, then increased to 2.0 liters per 6 minute cycle for residue clearance. A 16-hour light cycle was regulated with a 24-hour timer. Stock solutions of the toxicant were prepared weekly and the mean specific activity, used for computations, was 91.65 pCi/µg. Carbon-14 labeled BAS 352 F was delivered with a Micromedic Systems Automatic Dipette with dual injector pumps.

Surviving fish were moved to the depuration chamber on day 30. Samples of water and fish were obtained from various days of both test periods. Tissues were oxidized and then counted on a liquid scintillation counter.

REPORTED RESULTS:

A. Radioactive Residues in Water

The mean values for two water samples from each day assayed are presented in Table 1. The mean concentration for the uptake phase was 0.456 ppm. The concentration of carbon-14 residue from day 7 of the depuration period to termination was below the minimal detectable concentration (0.009 ppm). Table 3 shows control water residues (means of two samples). Values are of background magnitude.

B. Radioactive Residues in Fish Tissue

Values in Table 2 are means of three samples of whole fish and means of ten replications of each edible and nonedible portion.

Whole fish, edible tissue, and nonedible tissue showed maximum residue concentrations on day 14 of the uptake period. Whole-fish residues remained at a relatively stable concentration through day 7 whereas residues in edible and nonedible samples increased steadily through day 7. The reviewer questions the apparent contradictory results. The bioconcentration factor ranged from 107 to 241 in whole fish, 47.4 to 106 in edible tissue, and 198 to 317 in nonedible tissue. The mean bioconcentration factor for nonedible tissue was 3.19 times greater than the mean value for edible tissue and 1.48 times greater than the mean factor for whole fish. Day 14 of the depuration phase shows a decrease in the residues to 2.3% for whole

fish, 3.2% for edible tissue and 2.9% for nonedible tissue, of the maximum concentration during uptake.

Table 4 presents values for control fish; graphs 1 and 2 show residue levels for water, whole fish, and tissues.

C. Biological Observations

Bluegills exhibited a decrease in response to audio and visual stimuli, after three days exposure to BAS 352F. Feeding response was not affected. Normal reaction to sight and sound stimuli was regained after 96 hours in depuration water and surviving fish were in good physical condition at the end of the testing period. Total mortality was two fish from the control group.

DISCUSSION:

1. The objectives of the study consist of three parts. The first part was to determine the rate and extent of bioconcentration of carbon-14 residues in bluegill sunfish. Maximum uptake was exhibited on day 14, for all tissue, with the bioconcentration range presented in the results. The maximum bioconcentration factor for each tissue occurred on day 22.

2. The second part of the objective was to determine the distribution of concentrated carbon-14 residues between edible and nonedible portions of bluegill sunfish. The highest accumulation was found in nonedible tissue and the lowest in edible tissue. The mean bioconcentration was 262 for nonedible tissue, 176 for whole fish, and 82 for edible tissue.

3. The final part of the objective was to determine the rate and extent of carbon-14 residue elimination from bluegill sunfish placed in clean water. Lowest residues in all tissues were found on the last day of the depuration period. Elimination from maximum uptake value was 97.7% for whole fish, 96.9% for edible tissue and 97.1% for nonedible tissue.

VINCLOZALIN Sh# 113201

Page_____ is not included in this copy.

Pages 94 through 99 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

CHEMICAL: Ronilan, Vinclozolin, 3-(3,5-dichlorophenyl)-5-ethenyl-5methyl-2,4-oxazolidinedione (formerly BAS 353F - BAS 352 (04)F, 83 258)

FORMULATION: Technical

CITATION: Portnoy, C.E. and J.R. Clark (1978). Identification of ^{14}C Residues in Water and Fish from the BAS 352 F-^{14}C Bluegill Sunfish Bioaccumulation Study (unpublished report prepared by BASF Wyandotte Corp., NJ, Laboratory Report No. PM-22)

TRADE SECRET CLAIM: Yes

REASON FOR REVIEW: Registration of Ronilan

REVIEWED BY: S. Gould, Staff Scientist, Enviro Control, Inc. Rockville, MD

DATE OF REVIEW: August 24, 1979

TEST TYPE: Aquatic Accumulation Study

CONCLUSIONS:

1. Extractable residues of BAS 352F and its metabolites ranged from 62 - 79% in water, edible and non-edible fish tissues, and were identified in this study.

2. Total radioactive residues found in fish tissues were greatest (58.7 ppm for edible and 179.3 ppm for nonedible) on day 14 of the uptake phase but decreased to 4.6 ppm for whole fish on day 14 of the depuration phase.

3. The number of fish utilized in the study and the number used to determine residues were not presented. It is the reviewer's opinion that before data can be properly interpreted, the number of fish tissues analyzed must be known.

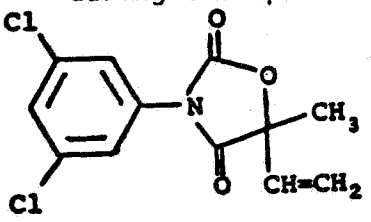
4. Flow-through protocol was not included in this study, although analytical methods appeared appropriate. Therefore, scientific validity cannot be determined.

15

100

MATERIALS AND METHODS:

A flow-through bluegill sunfish bioaccumulation study was conducted using BAS 352F labeled with carbon-14 in the phenyl ring at a concentration of 0.50 ppm. The test consisted of a 30-day uptake exposure and a 14-day depuration period. Water samples were taken during the uptake and depuration phases.



BAS 352 F - Vinclozolin.
3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-1,3-oxazolidine-2,4-dione

Extractions: Water was extracted with ethyl acetate followed by radioassay of the extractable and aqueous phases. Fish tissue was homogenized and extracted with methanol and partitioned into acetonitrile and hexane phases. Each phase was radioassayed. Portions of the residue were combusted to determine radioactivity not extracted by methanol. Total radioactive residues of fish tissue were combusted and counted.

Characterization of Radioactive Residues: TLC of extractable residues was carried out on silica gel plates using two solvent systems. Non-radioactive standards were located by UV light and radioactive compounds with a Berthold Thin Layer Scanner, a Birchover Spark Chamber, and by autoradiography. Quantitation was performed by counting on a Packard or Beckman Liquid Scintillation Counter.

REPORTED RESULTS:

A. Total Radioactive Residues

The residues of BAS 352F labeled with carbon-14 in the phenyl ring found in water were assayed at 331 ppm to 0.508 ppm during the uptake phase. The depuration period range was 0.001 ppm to 0.047 ppm. (Table I). Edible fish residues (Table II) showed a peak of 58.7 ppm on day 14 of the uptake period, declining to 50.5 ppm on day 30. Whole fish residue dropped to 4.6 ppm the last day of the depuration period.

B. Extraction and TLC Analysis of Water and Fish Tissue

Major residues found in ethyl acetate extracts of water samples by comparative thin-layer chromatography were metabolite B, BAS 352 F, and metabolite E, in decreasing order of magnitude. Residue analysis of fish tissue identified the major compounds as BAS 352F and metabolite F. Two unknown residues were also found, located near metabolite F with solvent system B-ethyl acetate/acetic acid/water (8/1/1). Metabolite B,BAS 352F, and metabolite E were located with solvent A-chloroform/acetic acid (95/5).

DISCUSSION:

1. The experimental conditions were not presented. Missing data included the number of fish in the study and the number of tissues analyzed. Raw data were notincluded. However, autoradiographs with scans were provided.

2. The concentration of the test material (0.50 ppm) was not related to the intended application rate. There was no mention of the actual dosage rate.

3. The TLC clean-up of the acetonitrile partition phase was thorough. Repeated analyses using two solvent systems allowed adequate separation of BAS 352F, metabolite F and unknowns 1 and 2.

4. Residue data from water samples show 27.5% of extractable radioactivity from day 30 were not characterized. However, the radiochromatographic scan does not indicate any area unaccounted for to be more than 10%.

5. Residue data from fish samples indicate more than 20% of extractable radioactivity to be unaccounted for. Radiochromatographic scans do not show any areas unidentified to be greater than 10%.

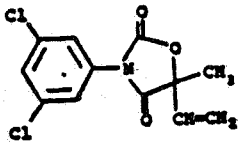
6. Unextractable residues from fish extraction with methanol increased from 7.4% on day 7 to 18.5% on day 30, in the edible portion. There was no explanation provided by the author.

7. The amount of unknown 1, found on day 7 in the edible portion of fish tissue, is close to 10%. This compound may need further identification.

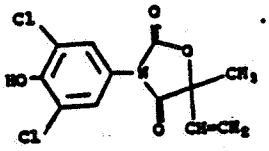
FIGURE I

standards

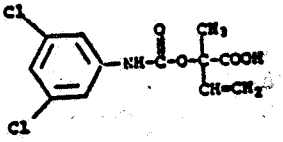
The following analytical standards were used for reference purposes and were obtained from Dr. Ohnsorge, BASF AG, Limburgerhof, West Germany:



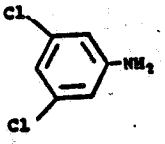
BAS 352 F - Vinclozolin
3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-1,3-oxazolidine-2,4-dione



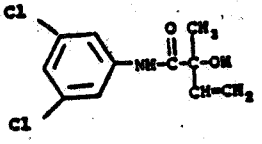
Metabolite A
3-(3,5-dichlorophenyl-4-hydroxyphenyl)-5-ethenyl-5-methyl-1,3-oxazolidine-2,4-dione



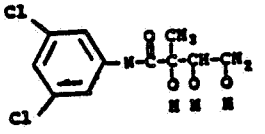
Metabolite B
3,5-dichlorophenyl-carbamic acid-(1-carboxy-1-methyl)-2-propenyl ester



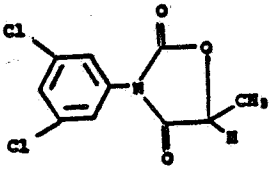
Metabolite D
3,5-dichloroaniline



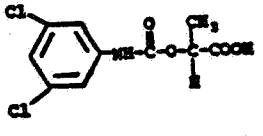
Metabolite E
N-(3,5-dichlorophenyl)-2-hydroxy-2-methyl-3-butenoic acid amide



Metabolite F
N-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxy-butanoic acid amide



Metabolite S
3-(3,5-dichlorophenyl)-5-methyl-1,3-oxazolidine-2,4-dione



Metabolite T
3,5-dichlorophenyl-carbamic acid-(1-carboxy)-ethyl ester

TABLE I

Total Radioactive Residues in Water Samples from the BAS 352 F-^{14}C Bluegill Sunfish Bioaccumulation Study

Days Since Initiation of Experiment	ppm[a]	Days Since Initiation of Depuration Period	ppm[a]
0	0.480	0	0.017
1	0.360	1	0.047
3	0.435	3	0.021
7	0.472	7	0.003
10	0.508	10	0.001
14	0.505	14	0.003
22	0.331		
30	0.417		

[a]Expressed as BAS 352 F-^{14}C equivalents

TABLE II

Total Radioactive Residues in Bluegill Sunfish from the BAS 352 F-^{14}C Bioaccumulation Study

Days Since Initiation of Experiment	Fish Tissue	ppm[a]
7	edible	41.3
	nonedible	145.9
14	edible	58.7
	nonedible	179.3
30	edible	50.5
	nonedible	138.2
44[b]	edible	2.2
	nonedible	5.4
	whole fish	4.6

TABLE III

Extraction and TLC Analysis of Water Samples from the BAS 352 F-^{14}C Bluegill Sunfish Bioaccumulation Study

Days Since Initiation of Experiment	% of Total ^{14}C Found in Water					
	^{14}C Extractable with Ethyl Acetate	Unextractable ^{14}C	Distribution of ^{14}C in Ethyl Acetate Extract			
			352	E	B	Origin*
7	96.9	3.1	26.5	9.3	42.8	2.3
10	95.3	3.8	27.2	10.6	40.1	6.8
14	91.8	2.5	20.3	10.1	46.7	1.5
30	94.3	2.2	12.5	9.9	42.2	2.2

*includes Metabolite F

105

TABLE IV

Extraction and TLC Analysis of Bluegill Sunfish from the BAS 352 F-^{14}C Bioaccumulation Study

Days Since Initiation of Experiment	Fish Tissue	% of Total ^{14}C Found in Fish Tissue							
		^{14}C Extractable with Methanol	Unextractable ^{14}C	Acetonitrile Partition Phase	Hexane Partition Phase	Distribution of ^{14}C in Acetonitrile Partition Phase after TLC Clean-up*			
						352	F	Unknown 1	Unknown 2
7	edible	94.7	14.0	92.7	1.1	35.7	23.5	9.5	2.5
	nonedible	93.8	6.6	90.6	1.6	61.9	6.3	3.0	0.9
14	edible	92.1	7.4	91.4	1.2	37.3	17.5	6.0	2.3
	nonedible	92.7	6.4	90.4	1.5	51.7	7.2	2.8	0.7
30	edible	92.4	18.5	91.2	1.3	34.0	24.7	6.2	2.9
	nonedible	88.8	19.7	82.5	3.6	52.6	9.3	2.6	1.1

*An additional 8-14% of the total ^{14}C in each sample was distributed over the three TLC plates used for each analysis as unidentifiable material.